EFFECT OF SODIUM CHLORIDE ADDITION DURING DIAFILTRATION ON THE SOLUBILITY OF MILK PROTEIN CONCENTRATE

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ABSTRACT

EFFECT OF SODIUM CHLORIDE ADDITION DURING DIAFILTRATION ON THE SOLUBILITY OF MILK PROTEIN CONCENTRATE

Scott Joseph Gualco

There is considerable interest among food manufacturers to incorporate protein into food products in both developed and developing countries. Dairy proteins are excellent choices for many different applications, as they are known to have several nutritional and functional benefits. Membrane filtration techniques are often utilized as the preferred method of fractionation, due to the high throughput and continuous nature of the process. One such product produced from membrane filtration of skim milk is called milk protein concentrate. This product is valued for its high protein content, but it has historically exhibited poor solubility when reconstituted into water, which severely restricts the food applications for which it is suitable. There is some existing evidence that milk protein concentrates which contain elevated levels of sodium exhibit higher solubility upon reconstitution into water. The main objective of this thesis project was to demonstrate the effect of sodium chloride, added to diafiltration water utilized during the manufacturing process, on the solubility of milk protein concentrate.

It was observed that the addition of sodium chloride into diafiltration water at levels of 50 mM, 100 mM, and 150 mM had a beneficial effect on the solubility of milk protein concentrate across a variety of reconstitution conditions. For example, when milk protein concentrate was mixed for 1 h on a stage mixer at 23 °C ± 1 °C, a significant increase ($p < 0.001$) in mean solubility was observed when at least 50 mM NaCl had been incorporated into DF water. The incorporation of 50 mM NaCl into DF water

significantly increased ($p < 0.001$) the mean solubility of milk protein concentrate from 59.81 % to between 64.34 % and 71.78 %. The addition of 100 mM NaCl significantly increased ($p < 0.001$) the solubility to between 88.80 % and 96.24 %, and the addition of 150 mM NaCl significantly increased ($p = 0.005$) the solubility to between 92.79 % and 100 %.

Minerals analysis of dry powders revealed a significant increase ($p < 0.001$) in levels of sodium. The addition of 50 mM NaCl into DF water was associated with a significant increase ($p < 0.001$) in powder Na content to between 2.48 mg/g and 7.44 mg/g. The addition of 100 mM NaCl into DF water was associated with a significant increase ($p = 0.002$) in powder Na content to between 5.80 mg /g and 10.75 mg/g, and the addition of 150 mM NaCl into DF water was associated with a significant increase ($p = 0.001$) in powder Na content to between 9.57 mg/g and 14.53 mg/g. A significant difference ($p < 0.001$) in magnesium level was also detected. Differences in calcium content were not found to be statistically significant ($p = 0.016$) at $\alpha = 0.01$.

Preliminary observations of milk protein concentrate upon reconstitution were made using a confocal laser scanning microscopy method. This method showed evidence of possible differences in powder particle rehydration and affinity for lipid association between powder particles manufactured at different treatment levels. As the level of NaCl incorporated into DF water increased, particle structures upon rehydration appeared more porous, and the incidence of lipid material that was not associated with powder particles appeared to increase. Overall, this study demonstrates the importance of sodium content in determining the solubility of milk protein concentrate.

Keywords: milk protein concentrate, solubility, sodium, calcium

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1. INTRODUCTION

As the demand for high-protein food products increases, food manufacturers have desired to incorporate protein into many different food systems. Dairy proteins are excellent choices for a variety of food applications because they have several benefits from a nutritional and functional perspective. Membrane filtration is one of the most efficient ways of separating dairy proteins on an industrial scale. One relatively new product is produced from membrane fractionation of skim milk, and this product retains the technologically and nutritionally important dairy proteins. The retained fluid can be incorporated in liquid form into a food product, or can be dried to powder form to facilitate storage, transportation, and incorporation at a later time. The fluid and dried powdered forms contain the same proteins in the same proportions as are present in skim milk, and have been utilized in recent years as a functional ingredient in the food industry. The dried form is commonly referred to as milk protein concentrate (MPC).

Researchers are striving to understand and improve the functional characteristics of MPC as the market demand for this ingredient increases. This high-protein dairy powder has historically exhibited poor solubility when reconstituted into water, which severely restricts the food applications for which it is suitable. MPC must be dissolved in water before it can express most desirable functional properties. Existing studies have indicated possible connections between mineral content and solubility of MPC. To further investigate these links, we propose a study examining the connection between MPC solubility and an MPC manufacture process utilizing the incorporation of NaCl at various levels. This study will also investigate the changes in MPC physical characteristics that occur as a function of varying the NaCl levels utilized during this

manufacture process. This may provide a better understanding of the relationship between MPC manufacture process, MPC composition, MPC physical characteristics, and solubility.

2. LITERATURE REVIEW

2.1. Brief Outline

This literature review aims to establish a common foundation of knowledge in the area of milk protein concentrates (MPCs) with respect to solubility, a key functional property that is poorly expressed in MPCs containing greater than 70% protein. This review will first examine the highly variable composition of MPCs available both domestically and internationally, while paying special consideration to correlations between select mineral constituents and solubility. The effect of manufacture process on solubility will be discussed, along with the chemistry governing interactions between calcium, sodium, and proteins in the MPC system. Finally, a series of experiments are proposed to more thoroughly investigate the observed links between composition, manufacture process, and solubility of the resulting MPC.

2.2. Definition of Milk Protein Concentrate

MPC can be defined as a group of products with varying bovine milk protein content produced from the ultrafiltration (UF) of skim milk and subsequent water removal (Puhan, 1990). UF may include one or more diafiltration (DF) steps to remove lactose, minerals, and water. Further water removal may involve reverse osmosis, vacuum evaporation, and spray drying. The resulting powder expresses unique physical, chemical, and functional characteristics when dissolved in solution, due to both the high concentration of proteins present and the thermal conditions imposed upon these proteins during processing (Tong, 2007). A typical MPC manufacture process is illustrated in Figure 1.

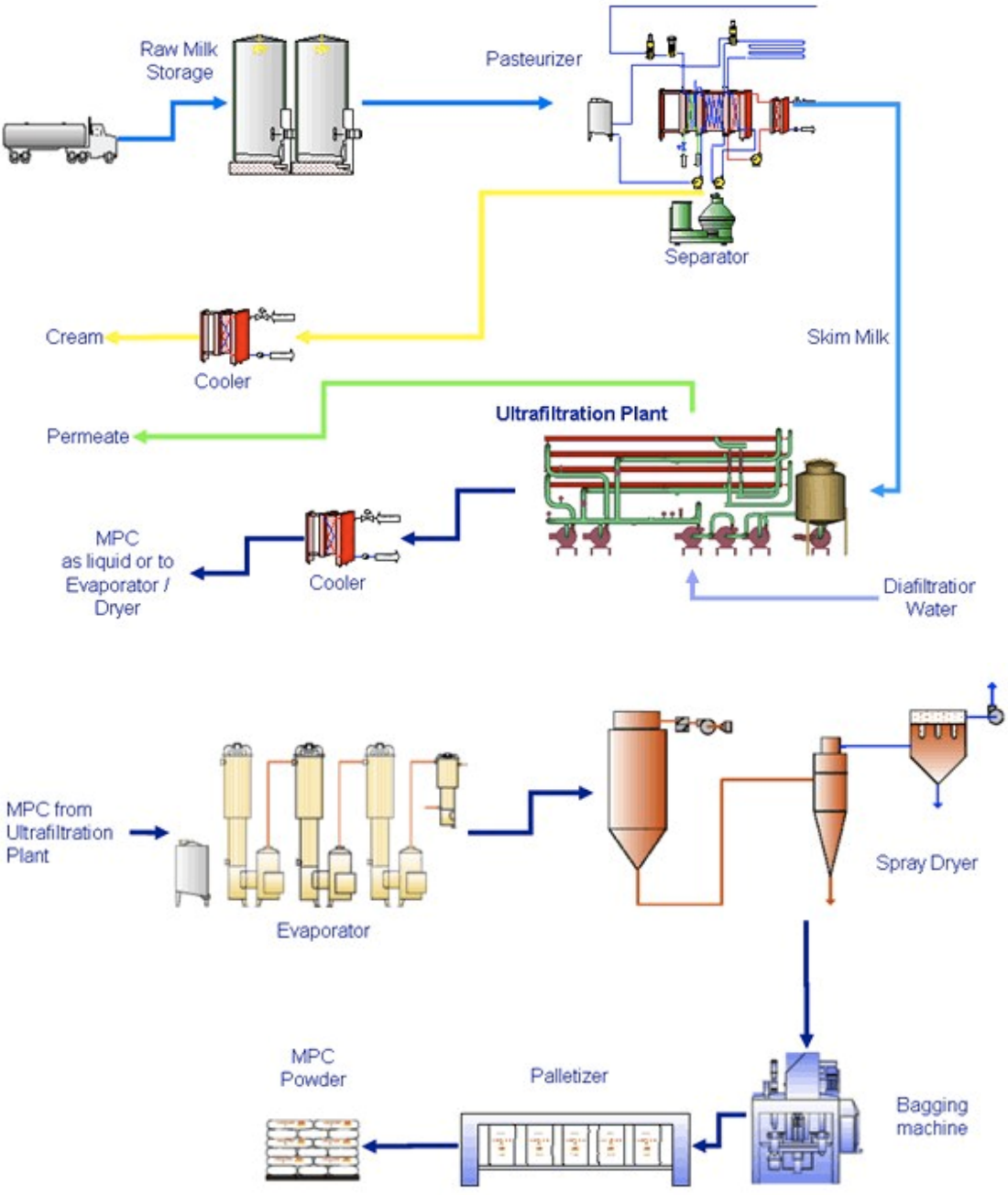

Figure 1: Typical MPC manufacture process from raw milk acquisition to powder storage, from Anonymous (2010b)

Neither the Food and Drug Administration nor the Codex Alimentarius Commission defines MPC. As such, Chapter 4 of the Harmonized Tariff Schedule of the United States (HTSUS) is most frequently cited as the quality standard to which U.S. food manufacturers hold all MPCs. According to Chapter 4 of the HTSUS, MPC is classified as "any complete milk protein (casein plus lactalbumin) concentrate that is 40 percent or more protein by weight." Two categories of imports are classified under the HTSUS. Moreover, in 2001 nearly all MPC utilized in the U.S. food industry was imported from New Zealand, Australia, Ireland, Germany, and the Netherlands, and MPC manufacturers needed only to meet the HTSUS standard that fits the category of import (Bailey, 2003).

MPC is most often manufactured using ultrafiltration and subsequent water removal processes. However, because the HTSUS only loosely defines MPC by composition, the HTSUS definition is tolerant of both dairy-derived protein products produced through other manufacturing processes and dairy-derived protein products with a high degree of composition variability. Laboratory analysis has confirmed large discrepancies in composition amongst MPCs from U.S. market suppliers, along with vast differences in MPC functionality (Floris et al., 2007). It was suggested that these differences in composition were due to variations in processing conditions. However, there is insufficient published data to correlate MPC manufacturing process and composition of the resulting powdered product with expression of its functional properties important to food manufacturers.

2.2.1. MPC Composition

MPC is commercially available at various protein concentrations, and these products are labeled as MPCX, with X referring to the percent protein in that particular product. For example, MPC containing 40% protein is labeled MPC40, and MPC containing 80% protein is labeled MPC80. MPCs containing more than 85% protein are classified as milk protein isolate (MPI) (Tong, 2007).

Table 1: Composition of various MPC products by protein content (Anonymous, 2001).

Product	Origin	Protein %	Fat %	Ash %	Lactose %
MPC42	AU	42.0	2.0	8.0	45.5
MPC42	NZ	42.0	1.0	7.5	45.5
MPC50	AU	49.8	1.5	8.0	35.5
MPC56	AU	55.8	1.5	8.5	30.5
MPC56	NZ	56.0	1.2	8.0	31.0
MPC70	AU	69.8	2.0	8.5	15.5
MPC70	NZ	71.0	1.0	7.0	17.0
MPC75	AU	74.8	2.0	8.5	10.5
MPC80	AU	79.8	2.5	8.5	5.5
MPC85	AU	84.8	2.5	8.5	0.5
MPC90	NZ	86.7	1.6	7.1	1.0

Source: Adapted from (Anonymous, 2001).

2.2.1.1. Variation in MPC Composition and Solubility

Although MPC is most often produced using a combination of UF, DF, and spray-drying processes, there currently exists no standardized procedure for its manufacture. In addition to ultrafiltered milk products, HTSUS classification includes concentrates obtained by dry blending nonfat dry milk (NFDM) with whey protein concentrate (WPC) and/or casein (CN) products, or mixing condensed liquid skim milk with liquid WPC and subsequently spray-drying to achieve a dry protein product (Durant, 2002).

The wide variations in solubility between MPCs obtained from different sources has been documented. A study examining 37 different MPC products from 10 different countries found no correlation between protein content and solubility, save for a small subset of MPCs in the range of 82% to 86% protein (de Castro-Morel and Harper, 2002). Detailed proximate analysis indicates that vast differences exist in MPC composition and functionality amongst MPCs obtained from different manufacturers, even amongst MPCs concentrated to similar protein levels (Floris et al., 2007). Table 2 illustrates observed differences in protein, K, Ca, and Na content, along with solubility as determined by the Nitrogen Solubility Index (NSI).

This report contains a number of key observations that may provide insight for MPC manufacturers who desire to deliver products of consistent or improved quality. Samples 60X11x and 60X13 (Table 2), both highly soluble MPCs containing greater than 80% protein, possess higher Na content than other MPCs, indicating that they may have been produced using processes involving Na addition as an intermediary step prior to, after, or during the ultrafiltration or diafiltration steps. Samples 60X11 and 62X13 (Table 2) also contain relatively low amounts of Ca when compared to other samples, which indicates an intermediary step in which Ca was removed from the product, or at least an intermediary step designed to transfer calcium from colloidal phase to ionic phase so that it could be removed during the UF and DF processes.

Table 2: Composition data relating solubility of various MPCs to K, Ca, and Na content (Floris et al., 2007).

Sample code	Protein %	Origin	mg/g K	mg/g Ca	mg/g Na	Solubility (NSI %)
20A1	69.2	US	7.62	19.29	2.14	76
21A2	76.3	US	3.73	21.85	2.10	68
22A3	84.0	US	1.61	23.43	1.35	28
23A4	56.9	US	10.29	17.04	2.95	81
24A5	57.4	US	10.29	17.11	2.94	77
25A6	68.3	US	7.24	19.04	1.97	72
26A7	71.0	US	6.90	19.82	1.92	74
27A8	54.6	US	11.05	16.21	3.02	75
28A9	79.0	US	3.96	23.88	1.22	55
51X02	80.7	world	3.35	23.28	1.21	57
52X03	77.5	world	4.18	22.75	1.11	64
53X04	59.8	world	9.55	18.10	2.39	72
54X05	72.5	world	5.05	21.48	1.54	60
55X06	79.5	world	4.56	21.63	1.25	72
57X08	80.1	world	3.39	22.12	0.79	53
58X09	60.0	world	9.95	18.62	2.54	74
59X10	78.1	world	4.24	22.05	0.95	57
60X11	80.2	world	1.79	12.94	12.83	79
61X12	85.3	world	1.67	22.06	0.29	62
62X13	80.6	world	1.92	12.65	13.70	80
63X14	79.8	world	2.95	23.54	0.69	56
64X15	59.8	world	11.51	14.44	2.52	68
65X16	70.0	world	9.26	14.87	1.74	72
66X17	80.6	world	7.11	16.00	0.91	68
68X19	80.8	world	3.76	24.19	1.13	59
69X20	67.1	world	8.43	21.78	2.05	82
70X21	69.2	world	7.98	20.48	1.99	87
71X22	59.0	world	10.88	18.89	2.74	88
72X23	81.3	world	3.84	22.88	1.33	58
72X24	80.1	world	3.95	25.50	1.59	63
74X25	69.0	world	8.27	21.51	1.84	72

Source: Adapted from Floris et al. (2007).

It is possible that samples with low Ca content were manufactured using a skim milk acidification process prior to UF and DF. Such an acidification step may result in a shift in the location and state of calcium, from colloidal calcium phosphate to ionic calcium (Walstra and Jenness, 1984). Ionic calcium may later be removed by UF and DF processes. However, any pretreatment must take care to avoid separation of casein and whey components, and thus it is not desirable to obtain a complete isoelectric casein precipitation. More likely, an acidification process is utilized whereby skim milk is partially acidified to later aid in the removal of Ca (and later addition of selected minerals), but isoelectric precipitation is prevented from occurring by carefully controlling skim milk pH.

Unfortunately, since the manufacture process of each MPC examined in NIZO study is unknown, it is not possible to investigate any connections between manufacture process and solubility utilizing this data. Additionally, the study cannot provide information about the nature of processing variation that may occur during manufacture.

2.2.1.2. Correlation Between Mineral Composition and MPC Solubility

There is some evidence that increasing either Na or K content may improve the solubility of MPC, and both method and time of mineral addition may have significant impact on the solubility of the resulting powder. Carr et al. (2002) demonstrated that addition of monovalent salt to either skim milk (prior to the UF process) or final retentate (prior to spray-drying) could yield an MPC with enhanced solubility, though no explanation for the observed increase in solubility was reported. Dybing et al. (2007) discussed some principles of mineral manipulation in context of UF, and noted acidification of milk prior to UF could lower Ca content of MPC (most likely by shifting

Ca equilibrium towards the ionic phase, where it would then permeate through the membrane during the UF process).

An MPC manufacture process involving skim milk acidification was described in great detail by Moran et al. (2001). First, pasteurized skim milk was treated with an edible acid to obtain a pH between 5.9 and 6.5. Second, the skim milk was concentrated via UF to at least 15 % total solids. Third, the pH of UF retentate was adjusted to between 4.9 and 6.3 by the addition of edible acid. Finally, the retentate was dried by evaporation and spray-drying to obtain an MPC containing up to 70 % total solids. This MPC could more easily be incorporated into process cheese than such an MPC that did not undergo a manufacturing process involving acidification, and the reduced-calcium properties of this MPC were found to favorably impact the texture and functionality of the resulting processed cheese. A few disadvantages to this process, such as possible protein precipitation along with changes in retentate viscosity, UF flux, and Na contamination of permeate, were documented.

It is not known which, if any, of these processes are currently utilized to manufacture MPC products available in the U.S. A survey of MPCs available from commercial suppliers indicates that at least one supplier advertises an instantantized MPC with improved solubility characteristics (Anonymous, 2010a), and at least one supplier advertises MPC tailored for yoghurt applications (Anonymous, 2007). Upon request, most suppliers will attempt to tailor MPCs towards various food applications, suggesting that some data correlating composition or manufacture parameters to MPC functionality is held internally.

2.2.1.3. Implications of Observations Linking Mineral Composition and MPC Solubility

In milk, strong structural stability of proteins is imparted by electrostatic interactions between amino acid chains and ions in solution. Caseins contain several potential sites for ionic bonds between casein molecules, and it is believed that these sites play major roles in micelle sub-unit interactions (Farrell, 1988). Milk is sensitive to changes in ionic strength and pH; for instance, as pH approaches the isoelectric point of casein, inter- and intra-protein electrostatic interactions are increased to a point where casein precipitation occurs (Mulvihill and Fox, 1989b). Furthermore, there is great interest among researchers and technologists to best predict and utilize the ionic equilibria in milk to manufacture dairy products (Fox and Brodkorb, 2008).

The equilibria between ionic colloidal salts of milk can be altered by many factors (Fox and McSweeney, 1998). For example, acidification of milk brings about the progressive solubilization of colloidal calcium phosphate, as well as other colloidal salts, from casein. This solubilization is complete between pH 4.6 and 4.9. The addition of alkali is known to have the opposite effect. At roughly pH 11, nearly all calcium in milk can be found in the colloidal phase. Changes in temperature cause large shifts in the solubility of calcium phosphate (Fox and McSweeney, 1998). The solubility of calcium phosphate increases with decreasing temperature. Low-temperature (in the range of 20 °C to 3 °C) shifts in this equilibria are reversible, but reversibility decreases upon thermal treatment of milk.

Ionic equilibria play an important role in the properties of fluid milk, and it follows that ionic equilibria of dairy powders produced from processes utilizing milk may have a powerful ability to alter the solubility of those powders. Changes in ionic

equilibria are known to impact electrostatic interactions and hydrophobic interactions, two important factors dictating the solubility of a protein (Walstra et al., 2006).

The solubility of dairy proteins is also known to decrease upon storage, and this phenomenon has been shown to occur in MPC upon storage at high temperature (Anema et al., 2006). One possible mechanism for the observed decrease in MPC solubility upon storage involves the Maillard reaction. It was noted that significant lactosylation occurred in MPC85 samples, particularly on κ-CN, that had been stored for 7 days at 50 °C (Figure 2). Cross-linking of the proteins at the interface of the MPC85 powder were also implicated as a possible reason for the impaired solubility of the MPC upon storage. These findings were largely consistent with the findings of Mimouni et al. (2010), who provided field emission scanning electron micrograph evidence of intermicellar bridging still intact during rehydration.

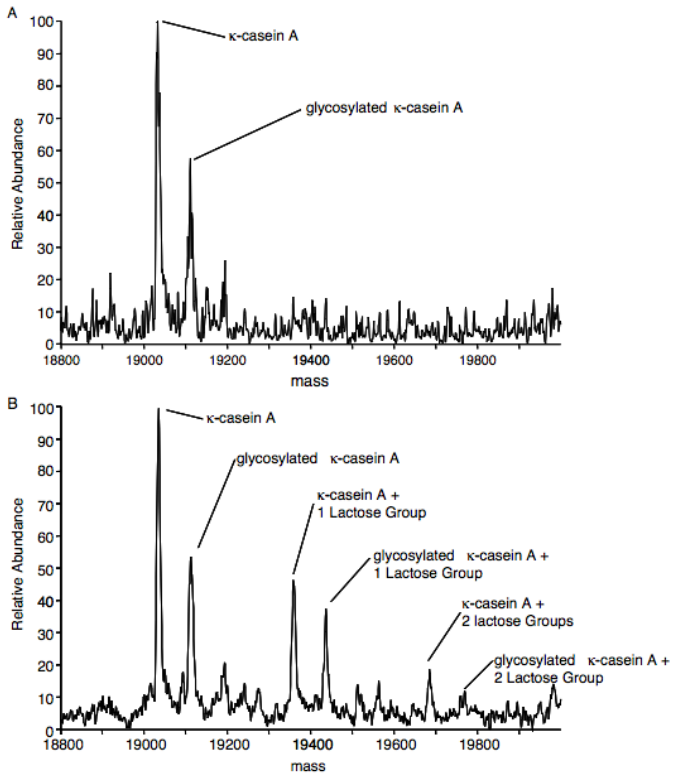

Figure 2: Mass spectrum of κ-casein A in untreated MPC85 (A) and MPC85 after 7 days of storage at 50 °C (B) Source: Adapted from Anema et al. (2006)

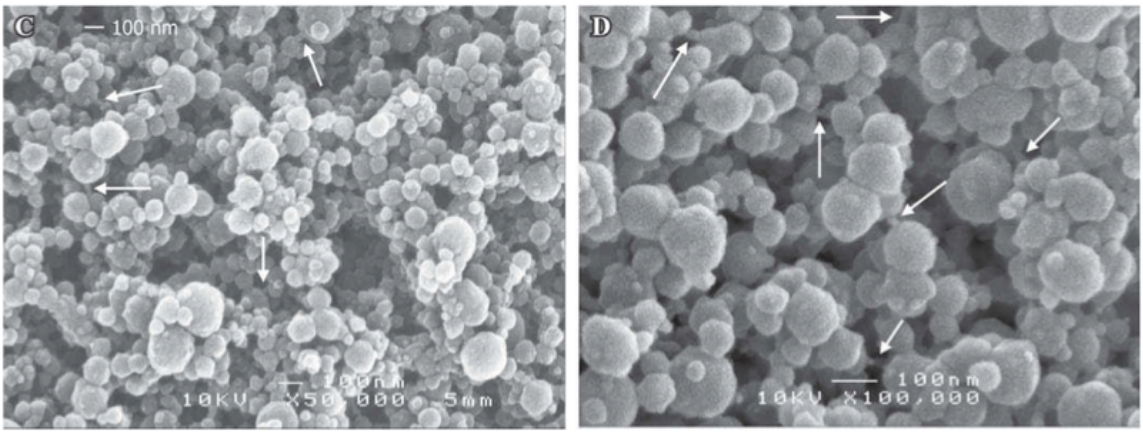

Figure 3: Details of the surface of rehydrated MPC85 powder particles at 2 magnifications (x50,000 [C] and x100,000 [D]). The white arrows in panels C and D indicate the presence of intermicellar bridges (Mimouni et al., 2010).

2.2.2. Proposed Model of Protein Aggregation During MPC Manufacture

The hairy layer of κ-casein chains impart casein micelle stability in the colloidal milk system through both electrostatic and steric repulsion, and it follows that if the hairy layer is removed, or if the hairy layer collapses, then a sufficient decrease in electrostatic repulsion occurs that can result in casein aggregation (Cho et al., 2003). Micelles depleted of κ-casein are also more prone to aggregation that those with intact κ-casein hairs on the micelle exterior (Walstra and Jenness, 1984). Casein aggregation can occur only under conditions in which it results in a lower free energy, which furthermore is due to a decrease in enthalpy (Walstra et al., 2006). When aggregation occurs, it causes a decrease of entropy, which in turn causes an increase in free energy (Equation 1), which simultaneously counteracts aggregation.

$$\Delta G = \Delta H - T\Delta S$$

Equation 1: Gibbs free energy equation for constant temperature, where ΔG is the change in Gibbs free energy, ΔH is the change in enthalpy, T is the absolute temperature, and ΔS is the change in entropy

The model of heat-induced coagulation of milk proposed by Walstra et al. (2006) describes two distinct series of reactions that may cause casein coagulation. The first, which is highly dependent on Ca^{2+} activity, is colloidal in nature and is not strongly dependent on temperature. The first series of reactions involves the formation of Ca salt bridges when colloidal interactions (also determined by the location of κ-casein and pH of the milk system) dictate that caseins come into close proximity with one another. The second series of reactions can be described by the formation of chemical cross-links.

These covalent interactions are strongly temperature-dependent. Covalent interactions may result in cross-links between or within peptide chains. Possible cross link formations, which may severely inhibit protein solubility, are shown below (Table 3).

It follows that, as caseins (along with whey proteins) are progressively concentrated during the UF process, and if distances between micelles are also decreased (Erdem, 2006) (and further discussed in Section 2.4.3.1), possibly also by way of decreasing electrostatic interactions due to mineral depletion and a net decrease in ionic strength, then the hairy layers of κ-casein on separate micelles may be more likely to come into contact with one another.

Table 3: Possible Reactions of Side Chain Groups of Amino Acid Residues Linked in the Peptide Chain (|) of Proteins at High Temperature

Reactant	Direction	Product
$\vert\text{—}CH_2\text{—}S^- + {}^-S\text{—}CH_2\text{—}\vert$ Cysteine	→	$\vert\text{—}CH_2\text{—}S\text{--}S\text{—}CH_2\text{--}\vert + 2e^-$ Cystine
$\vert{=}CH_2 + HS\text{—}CH_2\text{—}\vert$ Dehydroalanine, Cysteine	→	$\vert\text{—}CH_2\text{—}S\text{—}CH_2\text{—}\vert$ Lanthionine
$\vert\text{—}(CH_2)_4\text{—}NH3_3^+ + H_2C{=}\vert + OH^-$ Lysine, Dehydroalanine	→	$\vert\text{—}(CH_2)_4\text{—}NH\text{—}CH_2\text{—}\vert + H_2O$ Lysoalanine
$\vert\text{—}CH_2\text{—}COOH + H_2N\text{—}(CH_2)_4\text{—}\vert$ Aspartic Acid, Lysine	→	$\vert\text{—}CH_2\text{—}CO\text{—}NH\text{—}(CH_2)_4\text{—}\vert + H_2O$ Isopeptide
$\vert\text{—}CH_2\text{—}S\text{—}S\text{—}(CH_2)\text{—}\vert$ + $\vert\text{—}CH_2\text{—}S^-$	↔	$\vert\text{—}CH_2\text{—}S^-$ + $\vert\text{—}CH_2\text{—}S\text{—}S\text{—}CH_2\text{—}\vert$

Source: Adapted from Walstra and Wouters et al. (2006)

When two micelles closely approach (due to Brownian motion) the protruding κ-casein hairy layers may overlap, even though steric repulsion between micelles occurs at

a distance of roughly 20 nm. It is believed that cross-linking between reactive groups on κ-casein chains may occur, and that the number of linkages formed increases as the hairy layer interpenetration depth increases. Ca may play an integral role in forming initial short-lived bonds, which may be salt bridges between negatively charged groups. An excess of Ca ions may increase the probability of Ca bridge formation, particularly at κ-casein (despite only containing 1 to 3 PO_4 / mol) which may accelerate the rate at which Ca bridges between micelles may form.

At high temperatures, such as those in a typical spray-drying process, more long-lasting covalent bonds may form between amino acids if the necessary reaction radius has been penetrated, creating a strong network of many bonds termed a "junction." (Walstra et al., 2006). This heat also has the effect of gradually dissolving inorganic phosphate, causing the net negative electric charge of the casein micelles to decrease, favoring casein micelle aggregation. It is at this point that water is removed, leaving behind dried powder particles in which this junction remains intact. This junction may inhibit rates of water transfer when the final powder is introduced to water, leading to poor solubility of MPCs (Mimouni et al., 2010) as discussed in Section 2.2.1.1. The model proposed above may explain why an alteration in the ionic strength of the milk serum, involving the removal of Ca from retentates produced through a UF and DF process, may result in the creation of high protein powders which are more highly soluble than those that have not undergone a Ca removal process, as was observed previously (Dybing et al., 2007).

Yet, the question of how Na or K addition into an ultrafiltered retentate could yield a material, which upon subsequent diafiltration and spray-drying, which is more highly soluble than one which has not undergone Na addition, has not yet been fully

addressed. Caseins are unique proteins from a structural standpoint in that they contain repeating segments of serine phosphates (Fox and McSweeney, 1998). Caseins bind calcium at the ratio of about 0.016g Ca per g casein, or 0.4 g Ca per L milk (Demott, 1969). In addition, casein may bind Na and K at neutral pH, and this binding also occurs at phosphoserine groups of caseins. It is possible that the increased Na present in samples 60x11 and 62x13 and increased K in samples 23A4, 24A5, and others (Table 2) represents a state similar to that of sodium or potassium caseinates, which generally have better solubility than calcium caseinates. This is likely due to several factors, including a decreased net ionic strength (HadjSadok et al., 2008), increased electrostatic interactions, and decreased susceptibility to aggregate formation in sodium caseinate solutions as compared to calcium caseinate solutions. The role of Na in dictating the ionic strength of the milk solution, and subsequent changes that may be caused by ionic strength manipulation, will be discussed in Section 2.6.3.

2.3. Assessing the Current State of Solubility of MPCs with ≥ 70 % Protein

Though MPC has a variety of uses in dry-blending applications, a prerequisite for the expression of the majority of this ingredient's functional properties is ensuring the dispersion and solubilization of MPC. It can be generally stated that, although wide variation in MPC solubility has been previously observed, upon reconstitution at 20 °C of MPC containing ≥ 70 % protein, a portion of the solids remains undissolved and settles to the bottom of the container. Reconstitution of MPC containing ≥ 70 % protein is also typically characterized by poor dispersability of powder particles and impaired rate of hydration, which adversely impacts gelling, foaming, and emulsifying properties (Havea, 2006). It is also recognized that increased MPC storage temperatures result in a decrease

in solubility over time (Anema et al., 2006, Havea, 2006, Jimenez-Flores and Kosikowski, 1986, Mistry and Pulgar, 1996). In addition, it is clear that increasing the temperature of reconstitution results in a decreased amount of undissolved solids (Mistry and Hassan, 1991b, Mistry and Pulgar, 1996).

A variety of analytical work has been performed to understand the nature of insolubility of MPC containing ≥ 70 % protein. Havea (2006) noted the existence of only negligible differences between sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) patterns of MPCs (Figure 4), regardless of large differences in their solubilities. He also documented the presence of small amounts of disulphide-linked protein aggregates present on top of, and within, the stacking gel.

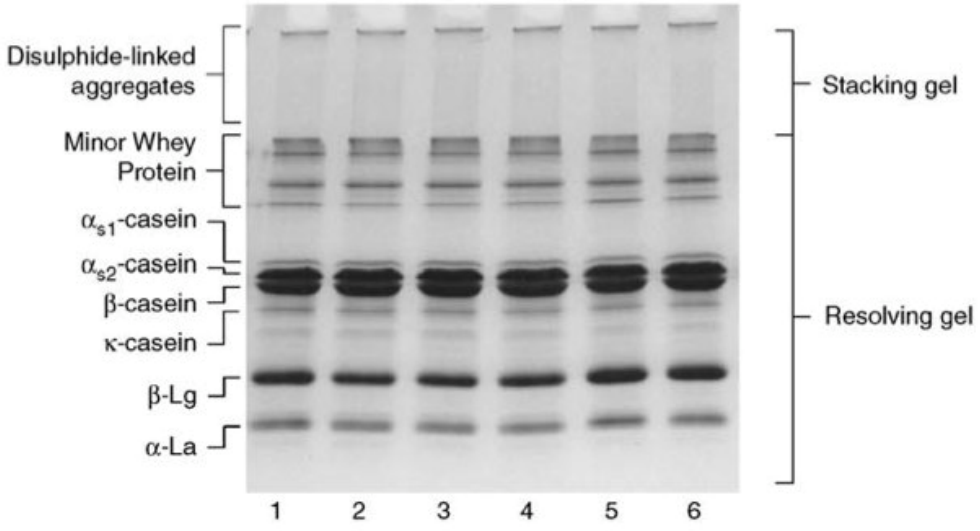

Figure 4: SDS-PAGE patterns of different MPC solutions observed by Havea (2006); lane 1: MPC85 with 53 % solubility; lane 2: MPC85 with 44 % solubility; lane 3: MPC85 with 41 % solubility; lane 4: MPC85 with 32% solubility, lane 5: MPC 85 with 96% solubility, lane 6: MPC with 98% solubility; protein concentration in samples ~ 1 g/kg, sample load = 10 µl. Gel image from Havea (2006)

He concluded that, based upon observed differences in alkaline-PAGE and SDS-PAGE electrophoretograms (Figure 5), hydrophobic association of casein molecules (with some apparent interaction with minor whey proteins) was present with the formation of insoluble material. It was also apparent from SDS-PAGE and Two Dimensional (2D) SDS-PAGE that disulfide bound aggregates were composed of β-lactoglobulin (β-LG) and κ-casein (κ-CN), as high-molecular weight protein aggregates moved to those respective locations in 2D SDS-PAGE, and resulted in more intense β-LG and κ-CN bands, respectively, in SDS-PAGE. Lastly, these disulfide bound aggregates most likely existed in a range of sizes, as they were present in both supernatant phases and sediment phases after centrifugation (700 x *g*, 10 min). There was no evidence to suggest that disulfide bound aggregates were responsible for the formation of insoluble particles.

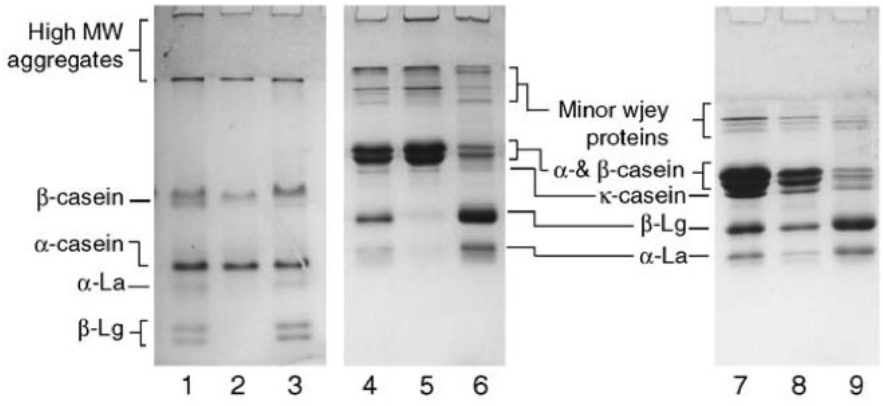

Figure 5: Alkaline-PAGE (lanes 1-3), SDS-PAGE (lanes 4-6), and reduced SDS-PAGE (lanes 6-9) patterns of 5% (w/w) MPC solution (41% solubility) as observed by Havea (2006); Lanes 1,4, and 7: MPC solution, lanes 2, 5, and 8: sediment; lanes 3, 6, and 9: supernatant; protein concentration in samples ~ 1 g/kg, sample load = 10 µl. Gel image from Havea (2006)

Havea's findings were consistent with those of McKenna (2000), who investigated the formation of insoluble material in MPC. Observations utilizing transmission electron microscopy (TEM) indicated that insoluble material consisted of particles up to roughly 100 μm in diameter. It was documented that these particles appeared to associate via some type of protein-protein interactions, and that an outer "skin" layer began to form on particles of MPC that were stored at 20 °C or higher, possibly inhibiting the movement of water into and between the particles.

It was also observed that CN dissociation from micelles can occur at pH 7.1 in heated reconstituted skim milk (Anema and Klostermeyer, 1997). Free CN, with a large amount of hydrophobic residues, may interact to form non-covalent linkages with non-dissociated CN during drying. It is probable that the formation of insoluble particles occurs during the drying process, but there is currently no data relating changes in casein micelle size and casein micelle mineral depletion that co-occur during the UF process, with the formation of such insoluble particles.

There is some data suggesting that significant changes in casein micelle size distribution occur during UF, which could induce a state in which hydrophobic interactions are more likely to occur upon drying (Srilaorkul et al., 1991). A kinetic approach suggested that heat-induced structural modifications of milk proteins are related to the concentration factor of the UF treatment, and found that the protein system in skim milk was reorganized to a progressively more compact structure via hydrophobic bonds as UF progressed. Heat treatment of this retentate may further induce a more "closed" casein structure, as surface hydrophobic sites of milk proteins bound with 1-anilinonaphthalene-8-sulfonate (ANS) became progressively unavailable for hydrophobic

bonding (Erdem, 2006). Despite this work, there is still limited knowledge of how micelles are affected by the MPC manufacturing process, and the potential effect of mineral modifications on both the retentate and dried powder.

It is important to view MPC solubility not only from the viewpoint of protein-protein or protein-water interactions, but also from a physical dispersion standpoint. Several authors have studied the rehydration characteristics of dairy powders using nuclear magnetic resonance relaxometry (NMR-R) methods, which provide data on the rehydration kinetics during powder reconstitution. It was found that, for native phosphocaseinates (NPCS), NMR data indicated changes in CN micelle structure, which most likely resulted from changes in protein-protein interactions that influenced hydration characteristics (Schuck et al., 2002). Other workers have utilized turbidity sensors to monitor the dairy powder rehydration process. These sensors are advantageous because they can distinguish between several phases of dairy powder rehydration, such as wetting (the process of a powder absorbing water when first contacting the water surface), sinking (the process of a powder sinking into the water), dispersing (the process of the powder separating into single particles throughout the water), and solubilizing (the process of rapid association with water and dissolution into water) (Freudig et al., 1999).

Use of turbidity sensors with NPCS solutions showed a clear ability to monitor the rehydration process, but further studies applying this approach to other dairy powders have not yet been done. It is likely that, if the introduction of sodium or potassium to skim milk retentate that has been produced via UF and DF processes changes the density or porosity of the resulting protein network of the MPC, changes in MPC rehydration and

rates of water transfer to the interior of the particles may be detected via the use of such turbidity sensors.

2.3.1. Methods of MPC Solubility Analysis

Solubility is defined as the property of a solid, liquid, or gaseous chemical solute to dissolve in a liquid solvent to form a homogeneous solution. Because MPC is a complex mixture of proteins, lipids, carbohydrate, minerals, and moisture derived from skim milk (which is itself not homogenous), dissolved MPC can never be considered truly homogenous. Therefore, it is prudent to consider more applicable definitions of solubility in context of MPC.

Mulvihill and Fox (1989a), define protein solubility as “the amount of protein in a sample that goes into solution or into colloidal dispersion under specified conditions that is not sedimented by low centrifugal forces." Existing standard methods of dairy powder solubility analysis conform closely to this definition, but often measure all solids that resist centrifugation, as protein material does not sediment to the exclusion of all other molecules. For example, the solubility index (SI) for nonfat dry milk, developed by the American Dairy Products Institute (ADPI), measures the amount of product sediment after the application of low centrifugal forces under specified conditions. This method is also used to measure the solubility of dry whey, dry buttermilk, and dry whole milk powder. The insolubility index (ISI) is a measurement of the ability of a powder to dissolve in water, defined as the volume of sediment in ml after centrifugation. It is typically used to analyze the solubility of skim milk, whole milk, and sweet buttermilk powder, but it may also be applied to other soluble, dried dairy products (Federation, 1988).

In literature related to MPC, solubility is defined using a multitude of different methods, though most methods cited or stated in literature conform to the definitions stated previously. For purposes of comparison of the effect of storage temperature on solubility, Anema et al. (2006) defined MPC solubility as:

$$s = \frac{(weight\ of\ supernatant\ dry\ material)}{(weight\ of\ bulk\ solution\ dry\ material)}\ x\ 100$$

Figure 6: Method used for determination of MPC solubility (Anema et al. 2006)

Solubility was calculated according to the equation in Figure 6 after mixing a 5% w/w MPC85 solution in MilliQ water with a propeller-blade attached to an overhead stirrer (while maintaining the temperature of the solution at 30 °C, for a total of 30 min), withdrawing a subsample of MPC solution, and obtaining the supernatant by centrifugation at 700 x *g* for 10 min. It was found that the solubility of MPC85 decreased exponentially with storage temperatures, and that insolubility of an MPC85 system may involve interactions in or between casein micelles that reduce the solubility of these macromolecular complexes.

For purposes of comparing the effect of processing methods involving monovalent salt addition on the solubility of MPC, the term "enhanced solubility" was defined only as "a product which on reconstitution into a 5% w/v solution provides less sediment on centrifugation for 10 minutes at 700 x *g* relative to the corresponding product" (Carr et al., 2002). It was observed that an increase in the cation to protein ratio of 0.035 to 0.100 moles per 100 g protein had a beneficial effect on the solubility of the

MPC, and the resulting MPC would be advantageous in the preparation of beverages and cheese manufacture.

Lack of a standardized MPC solubility analysis method compounds the difficulty in comparing data across multiple studies, as the method and temperature of reconstitution greatly impact the amount of material able to enter solution and resist centrifugation or sedimentation. Therefore, multiple solubility methods should be considered when conducting any experiment that aims to assess the effect of a manufacture process, or changes in that process, on the solubility of the resulting MPC. This minimizes the chance that one particular test "favors" MPC of a certain composition, particle structure, or rehydration characteristic.

2.3.2. Advances in Assessing MPC Solubility

In recent years, a number of workers have explored novel methods of characterizing MPC solubility. Such methods aim to analyze key characteristics that may rapidly and efficiently predict the solubility of MPC without the need of carrying out a formal solubility test, such as those discussed previously in section 2.3.1. The two methods described below have potential future uses in the quantification of MPC solubility.

The first method utilized fourier transform infrared (FTIR) spectroscopy in an attempt to correlate differences in MPC nitrogen solubility with changes in FTIR spectra (Kher et al., 2007). This work was able to correlate changes in nitrogen solubility of individual MPCs during storage with changes in spectra, and show sufficient evidence that second derivative spectroscopy and principle components analysis (PCA) in the amide I and II regions (1700 to 1400 cm^{-1}) and the fingerprint region (1800 to 700 cm^{-1})

could discriminate between MPCs which suffered loss of nitrogen solubility on storage, and MPCs which maintained consistent nitrogen solubility on storage.

The second method attempted to characterize the solubility of MPC using focused beam reflectance measurements (FBRM), which provides the ability to monitor changes in chord length over time under a variety of suspension concentrations (Fang et al., 2010). A characteristic dissolution profile for different MPC powders was able to be established, but it was recognized that further investigation must be conducted to confirm FBRM as a method suitable for MPC solubility analysis, especially given that MPCs vary widely in composition and dissolution characteristics.

Although both techniques could potentially be used in the future to better standardize and characterize the solubility of high-protein dairy powders, at present day they have not been adequately tested, and neither has been proven to assess the solubility of MPC in a way that can be traced across previous studies. Therefore, any current assessment of manufacture processes on the solubility of the resulting MPC should be conducted using existing methods of solubility analysis, such as those discussed previously in section 2.3.1.

2.4. Possible Changes in Casein Micelles During MPC Manufacture

2.4.1. Caseins

The casein micelles can be described as supramolecules, systems containing several molecular entities organized by noncovalent intermolecular binding interactions. Casein micelles represent roughly 80 percent of the total protein in bovine milk and other

commercial dairy species, and are the primary sources of calcium, phosphate, and protein for the mammalian neonate.

Table 4 lists characteristics that contribute to the biological purpose of milk and its ability to be utilized as a food ingredient.

Table 4: Average characteristics of casein micelles from bovine milk

Characteristic	*Value*
Diameter	120 nm (range: 50 - 500 nm)
Surface Area	8 x 10-10 cm^2
Volume	2.1 x 10-15 cm^3
Density (hydrated)	1.0632 g cm^3
Mass	2.2 x 10-15g
Water content	63%
Hydration	3.7 g H2O g^{-1} protein
Voluminosity	44 cm3 g^{-1}
Molecular Mass (hydrated)	1.3 x 109 Da
Molecular Mass (dehydrated)	5 x 10^8
No. of Peptide Chains	5 x 10^3
No. of Particle per ml Milk	10^{14} to 10^{16}
Surface of Micelles per ml Milk	5 x 10^4 cm^3
Mean Free Distance	240 nm

Source: Adapted from (Fox and McSweeney, 1998)

The stability and structure of casein micelles during processing is known to have strong implications on the properties of the final food product. Casein micelle structure has been the focus of exhaustive research (Dalgleish and Parker, 1980, Fox, 1989, Fox and McSweeney, 1998, Horne, 1998, Kruif, 1999, Sawyer, 1969a, Walstra and Jenness, 1984, Waugh and von Hippel, 1956). It is generally accepted that casein micelles exist as roughly spherical, porous entities, 50 nm to 500 nm in diameter, and there are several different models of casein micelle structure. These models fall into the general categories described as coat-core, internal structure, or subunit models (Fox and Brodkorb, 2008).

To date, the most accepted model is the submicelle model proposed by Walstra in 1984, which describes the casein micelles as composed of roughly spherical subunits called submicelles.

2.4.1.1. Effect of Temperature on Casein Micelles

The caseins are susceptible to temperature-induced association, which is largely a function of their high hydrophobic residue content, presence of highly charged segments, and cross-linking via organic phosphate groups. For example, α_{s1}-CN associates in a series of consecutive steps, which are dependent upon ionic strength and pH, but independent of temperatures below 30 °C. The association of α_{s2}-CN is dependent upon ionic strength, but is also relatively independent of temperatures below 30°C. However, the association of β-CN, in addition to being dependent on ionic strength, is highly temperature dependent (Fox, 1989).

A shift in temperature not only causes changes in milk protein association, but it also alters the ion-binding abilities of the individual caseins. Calcium-binding of the caseins is influenced by temperature, pH, and ionic strength (Dalgleish and Parker, 1980, Dickson and Perkins, 1971, Horne, 1998). At Ca^{2+} concentrations ordinarily found in milk, Ca^{2+} is bound to phosphoseryl residues, but at at high Ca^{2+} concentrations, binding to aspartyl or glutamyl residues may occur. This causes a reduction in protein charge along with significant changes in conformation, causing protein association and eventually precipitation to occur.

2.4.2. The Role of κ-CN on Micelle Structure and Stability

The isolation of κ-CN by Waugh and von Hippel (1956) drove casein micelle research towards the investigation of this particularly hydrophilic protein. κ-CN, a mixture of disulfide-bonded polymers, stabilizes and regulates the size of casein micelles, which can be considered a colloidal protein complex containing Ca^{+2} and inorganic P. Although it consists of only between 12 % to 15 % of the entire casein micelle system, κ-CN's hydrophobicity and solubility in the presence of Ca^{2+} contrasts to the relative hydrophobicity and susceptibility to Ca^{2+} -induced coagulation exhibited by the other casein variants. Not only is κ-CN insensitive to the presence of relatively high concentrations of Ca^{2+}, it can also stabilize nearly 10 times its weight of α_{s1}, α_{s2}, and β-CN against Ca^{2+} -induced precipitation. Thus, the location of κ-CN in relation to the other micellar proteins, and its ability to stabilize the casein micelle in the presence of Ca^{2+}, play a critical role in the structure of the casein micelle and the micelle's stability in the relatively calcium-rich milk system (Mulvihill and Fox, 1989a).

2.4.3. Ultrafiltration

If an increase in the degree of Ca-dependent colloidal interactions between κ-casein hairy layers is at least partly responsible for the formation of observed covalent bonds between proteins under conditions of heat treatment, then it is imperative that a clear understanding of how UF favors these interactions is needed. This cannot take place unless the basic principles of the UF process are first discussed.

UF describes a variety of membrane filtration processes in which hydrostatic pressure forces a fluid against a semipermeable membrane. Solids and solutes of high molecular weight are retained, while water and low molecular weight solutes pass

through the membrane into the permeate stream. In bovine milk, the general separation of components is illustrated by Figure 7. The efficiency of a membrane process is determined by both the membrane's selectivity, governed by its reflection, and the permeate flux. The rejection (R^*) of a particular solute *x* is described by the equation (Figure 8).

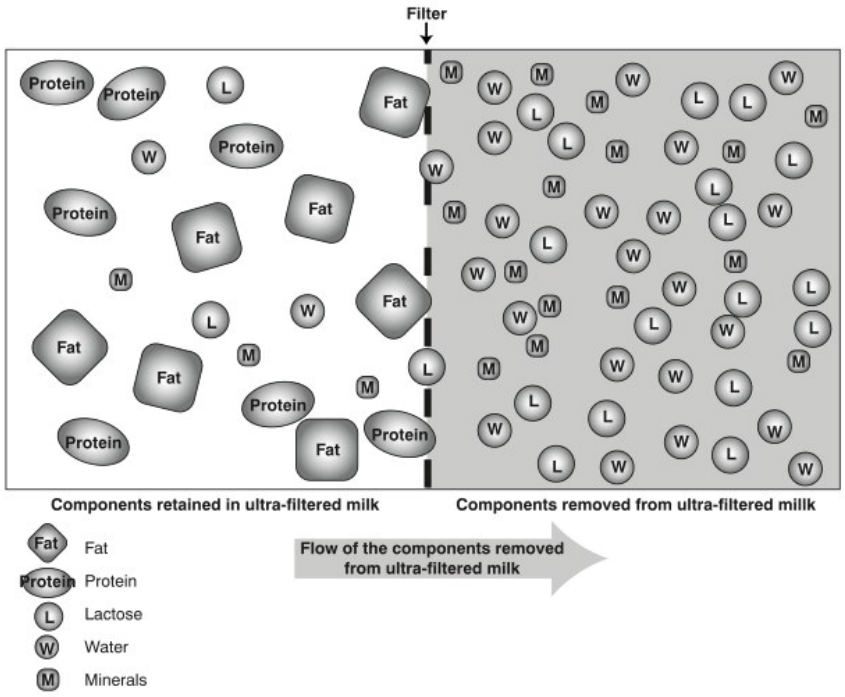

Figure 7: Flow of the components removed from ultrafiltered milk, from Anonymous (2001)

$$R^* \equiv \frac{q_w - \left(\frac{q_x}{c^*}\right)}{q_w} = 1 - \frac{q_x}{q_w c^*}$$

Figure 8: Description of rejection R* of a solute *x*, where q_w = flux ($kg \cdot m^{-2} \cdot s^{-1}$) of a solvent through the membrane, and q_x = flux ($kg \cdot m^{-2} \cdot s^{-1}$) of solute *x*, c* = concentration (kg solute / kg water) of *x* at pressure side of membrane

In ideal filtration situations, R* = 1 for all retained components, and R* = 0 for all components which permeate through the membrane. However, most components will have R* values between 1 and 0. Several factors in general are known to affect R*, including the type of membrane utilized, molecular weight of the solute, viscosity of the solute, concentration factor, and transmembrane pressure. With milk in particular, pH and temperature, which not only dictate solution viscosity but also alter the interactions between minerals and proteins, have an effect on both rejection and permeate flux, which is important in gauging the efficiency and speed of the UF process.

The permeate flux is described as the quantity q of liquid which passes through the membrane per unit time and surface area, and is defined by the equation (Figure 9):

$$q = \left(\frac{B}{h}\right)\frac{\Delta p}{\eta}$$

Figure 9: Description of the permeate flux of a membrane, where q = total permeate discharge, B = permeability coefficient of the membrane, h = effective thickness of the membrane, Δp = transmembrane pressure, and η = viscosity of the permeating liquid

There are several additional factors which effect the permeate flux during skim milk UF. For instance, protein molecules typically adsorb onto the membrane surface over the course of the UF process and reduce the effective pore width, reducing flux. In addition, at high flux values, a gel layer is compressed across the membrane surface which enhances membrane selectivity. High levels of calcium phosphate in retentate promote gel formation, and thus removal of calcium (for example by electrodialysis) is known to increase flux rates.

At typical operating pressures of 20 psi to 80 psi, the turbulent flow of the membrane surface prevents build up of particles and excessive membrane fouling (Pabby et al., 2009). UF membranes contain pores in the range of 0.1 to 0.001 μm. The typical UF membrane utilized by the dairy industry is a pressurized system containing a spiral wound module with negatively charged polyethersulfone membrane with a nominal molecular weight cut-off of 10 kDa. UF applications in the dairy industry include, but are not limited to, production pre-concentrated milk for cheese making, production of milk protein and whey concentrates and isolates, and acquisition of lactose and mineral rich permeates. It was estimated that over 300,000 square meters of membrane were installed in the dairy industry worldwide as of 1998 (Cheryan, 1998).

2.4.3.1. The Effect of UF and DF on Casein Micelle Size Distributions

The effect of UF on casein micelle structure is a topic of emerging research. Along with the previously discussed work of Erdem (2006), who observed the increasing hydrophobicity of the concentrated milk system during UF using the ANS hydrophobic probe, a number of authors have contributed observations to the area of casein micelle size changes as a result of UF. In highly concentrated UF milk retentate, caseins are allowed to interact over short distances, and this has shown to influence the size distribution of micelles. During UF, the distance between casein micelles was shown to decrease from approximately 120 nm to approximately 10 nm (Walstra and Jenness, 1984). Unfortunately, data relating casein micelle size to the UF process is not consistent across all studies. Some studies reported that casein micelle size appeared to be unchanged, while others reported increased or decreased sizes.

Karlsson et al. (2007) reported no observable changes in casein micelle size when UF retentate was observed under TEM, using three different preparation techniques, though it was must be noted that any TEM preparation (and virtually any microscopy preparation) may itself interfere with sample size. Singh (2007), utilizing electron microscopy, reported an increase in micelle size during the course of UF. It was also noted that micellar swelling occurred during DF, an increase in nonmicellar material was observed, and that the non-micellar material appeared to link together intact micelles, which is consistent with the findings of Srilaorkul et al. (1991). Meanwhile, Erdem (2006) observed decreases in micelle size as a result of UF. The most recent study was performed by Martin et al. (2010), who reported that casein micelles were not altered during the manufacture of MPC. This conclusion was reached by applying photon correlation spectroscopy (PCS) using the Cumulant method, also described in Martin et al (2007), to raw skim milks, UF/DF retentates, and concentrated UF/DF retentates. It was observed that differences in micelle size between these materials were less than 3 nm, and that this difference was not significant relative to the accuracy of the measurements. In addition, it was found that MPC reconstituted in water at 60 °C exhibited micelle sizes in the range of 210 nm to 197 nm within 30 minutes after reconstitution, to 197 nm to 195 nm after 1 h reconstitution, which is similar to size observed in skim milk in the same study and other studies of skim milk casein micelle size (Griffin and Anderson, 1983, Holt et al., 1973).

These results show that, in the determination of submicron particle sizes, PCS methods and other light-scattering techniques may be advantageous over microscopy methods because they do not require a sample preparation step that may alter sample

properties, and future work would benefit from the application of PCS to the study of ultrafiltered skim milk for this reason. The results of Martin et al. (2010), obtained by PCS methods, were consistent with those obtained through TEM by Karlsson et al. (2007) using three different sample preparation steps. Both studies were also consistent with (McKenna, 2000), who utilized electron microscopy and reported no change in casein micelle size distribution under conditions of UF. This body of work is also in agreement with the work of Montero (2010), who utilized dynamic light scattering (DLS) to observe the size of casein micelles at conditions up to 5X UF and noted no statistically significant difference in the size of casein micelles. Despite the earlier microscopy-based work of (Walstra and Jenness, 1984) and Singh (2007), the general agreement of PCS, TEM, and DLS methods indicates that casein micelle size is probably not affected to a significant degree as a result of UF. It is clear from the above research that microscopy-based methods alone, with the possible exception of TEM, cannot be used to draw accurate conclusions about the effect of UF on casein micelle size.

2.4.4. Evaporation and Spray-drying

The purpose of evaporation is to remove as much water as is practically possible without decreasing the quality of the final MPC. After UF (and, optionally, DF) the retentate is evaporated to remove additional water, and then spray-dried.

Spray drying is a method of producing a dry powder from a fluid by rapidly drying with hot air. All spray-driers contain an atomizer (spray nozzle) which disperses the liquid into droplets of uniform size, most commonly between 100 μm and 200 μm. The dried product is then collected in a drying chamber, and moisture-laden air is separated from dry powder using a cyclone separator.

Although the varying physical attributes (and to a lesser extent, the chemical attributes) of the resulting MPCs are relatively well documented, there has been little work examining the specific effects of spray-drying on casein micelles in milk, and even fewer studies have examined the specific effects of spray-drying on casein micelles in UF concentrated milk (Singh, 2007).

2.4.4.1. Influence of Spray-drying on Characteristics of Powder Particles

Valued technological properties of dairy powders are dictated by interactions between particles and water, such a rehydration and solubilization (Gaiani et al., 2005, Gaiani et al., 2007), particles and air (oxidation) (Keogh et al., 2001), and particle-particle interactions (flowability and stickiness) (Kim et al., 2005). These interactions are influenced by the chemical composition of powder particles, their morphological and physical properties, and their surface composition and structure. All of these characteristics can be altered by adjusting the various parameters of the spray-drying process.

A number of authors have published work providing insights to the changes in morphological properties which can occur due to variations in processing conditions and storage, which were found to influence surface composition and lipid distribution in several studies (Buma, 1971, Kim et al., 2002). With the application of X-ray photoelectron spectroscopy (XPS) to particle analysis, spray-drying conditions have recently been implicated as having profound effects on particle surface composition and topology. In a study performed on commercial native micellar casein (NMC) and commercial native whey isolate (NWI) powders, Gaiani et al. (2010) found that as outlet air temperature increased from 70 °C to 150 °C, the percentage lipids on the particle

surface decreased from 5.3 % to 0 %. When NMC contained lactose (added prior to drying), the percentage lipids on the particle surface decreased from 8.9 % to 0 % as spray-drying outlet air temperature increased from 70 °C to 150 °C.

The surface composition of native whey isolate (NWI) powders containing whey proteins were found to vary strongly with spray-drying outlet temperature. Lipid surface content of NWI particles decreased from 33.8 % to 10.6 % as spray-drying temperature increased from 70 °C to 150° C. When NMC contained lactose (added prior to drying), the lipid surface content of particles decreased from 27.6 % to 3.8 % as spray-drying outlet temperature increased from 70°C to 150°. Powders containing an 80/20 mix of casein to whey were also found to be affected by spray-drying outlet temperatures; lipid surface content of these casein/whey mixtures decreased from 10.1 % to 0 %. These results indicate that spray-drying outlet temperature is a critical factor which can influence the surface composition of powder particles in NMC, NMC containing lactose, WMI, and WMI containing lactose. It can be expected that spray-drying outlet temperature may impact the surface composition of MPC powder particles in a similar fashion, but given the relatively large amounts of lipids in some MPCs compared to the NMC and WMI powders studied previously, there is not sufficient data to hypothesize how proteins, lipids, and lactose may redistribute upon spray-drying at different outlet temperatures. Therefore, because the manufacture of MPC on large-scale commercial level requires the use of a spray-dryer, care should be taken to control such variables as inlet and outlet temperatures, feed rates, and ambient air conditions to minimize variation in overall MPC quality.

2.4.5. Possible Surface Formation Mechanisms

Previous research has suggested two main theories of surface formation of a drying droplet exiting the spray-drier nozzle. The first theory, supported by the work of Fäldt and Bergenstahl (1994) and Nijdam and Langrish (2005), contends that protein preferentially absorbs to the air/liquid interface on droplets, and that fat droplets must thusly migrate to the interior of the droplet. This theory is based on XPS measurements to analyze the chemical composition of spray-dried dairy based model emulsions. The work of Fäldt (1995) demonstrated that solutions containing surface-active components (such as proteins) tend to dominate the surface of a spray-dried powder particle. This observation was explained by a scenario in which the high surface activity of protein will absorb preferentially to the air/liquid interface of a droplet during atomization of liquid feed into hot air.

The second theory, supported by the work of Kim et al. (2003) and Kentish et al. (2005), based on slab drying experiments with a food model system containing water, sucrose, and sodium caseinate, takes into account the differences in binary diffusion coefficients of the spray-dried molecules. Because the binary diffusion coefficient in an aqueous solution can vary significantly between high molecular weight components and low molecular weight components, the transport velocity of each component towards the center of the droplet may be very different. It was observed that segregation of components does occur during spray-drying, and that the surface of powder particles could be expected to be enriched with components possessing the lowest diffusion coefficients (proteins and lipids). It was noted by Gaiani et al. (2010) that, at spray-drying outlet temperatures above 110 °C, the diffusion of components could be interrupted by

the solidification of the outer surface of the powder particle, and the subsequent immobilization of components trapped in that layer, before the system attains equilibrium.

Though these two theories shed some light onto the mechanisms of particle formation and the composition and structure of the resulting powder particles, further research must be conducted to understand the interactions of kinetic forces on particle formation (Kim et al., 2003). Little is known about the dynamic interactions which proceed during formation of droplets. In particular, the methods used by previous authors could be adapted towards the analysis of MPC, with little modification required, in order to further investigate the rehydration and solubility mechanics of these powders.

2.5. Other Milk Proteins

An understanding of the fundamental characteristics of milk proteins is integral when discussing the UF and DF of skim milk and the spray-drying of the resulting retentate; therefore this section will establish a common ground from which applied topics relevant to MPC manufacture can be explored.

2.5.1. Whey

The whey protein fraction consists of β-LG, α-lactalbumin (α-LA), and bovine serum albumin (BSA). The main whey protein is β-LG, which is 50% of the whey fraction and 12% of t otal milk protein (Fox and McSweeney, 1998). β-LG contains two disulfide bonds and one free sulfhydryl group that is located within its structure. This sulfhydryl group is revealed upon thermal denaturation, and it becomes available to react, particularly with κ-CN. The interaction between κ-CN. and β-LG involves both a

sulphydryl-disulphide interchange mechanism and non-covalent interaction (Sawyer, 1969b). Interactions between κ-CN. and β-LG are fundamental to all thermally-induced modifications of the functionality of milk proteins (Cho et al., 2003). The role of heat-induced κ-CN. and β-LG interactions in the formation of MPC insolubility is still under investigation.

α-LA comprises 20% of the whey protein in milk, and 3.5% of the total milk protein (Fox and McSweeney, 1998). α-LA contains four disulfide bonds. BSA exists in low concentrations; it is normally found in bovine blood, but transfers to milk in small amounts (Fox and McSweeney, 1998).

2.6. Minerals

Minerals are relatively minor constituents in bovine milk, but the ionic equilibrium they contribute to plays a significant role in determining the structure of the casein micelles. Bovine milk ordinarily contains salts in the quantities listed in Table 5. The total ionic strength of milk varies between 0.067 and 0.080. Ionic strength affects the electrical double layer thickness, which is about 1.1 nm in milk serum (Walstra et al., 2006).

Table 5: Distribution of milk salts in bovine milk

		Soluble		
Species	*Concentration (mg/L)*	*%*	*Form*	*Colloidal (%)*
Sodium	500	92	Completely Ionized	8
Potassium	1450	92	Completely Ionized	8
Chloride	1200	100	Completely Ionized	—
Sulphate	100	100	Completely Ionized	57
Phosphate	750	43	10% bound to Ca and Mg 51% H_2PO^- 39% HPO_4^{2-}	
Citrate	1750	94	85% bound to Ca and Mg 14% $Citr^{3-}$ 1% $HCitr^{2-}$	
Calcium	1200	34	35% Ca^{2+} 55% bound to citrate 10% bound to phosphate	66
Magnesium	130	67	Probably similar to calcium	33

Source: Adapted from Fox and McSweeny (1998)

2.6.1. Calcium

Ca plays an important technological role in the production of most dairy products, and it is of particular interest in MPC manufacture for reasons discussed previously in sections 2.2.1.3 and 2.2.2 Calcium in milk exists in three forms: Assuming a total Ca content ≈ 32 mM, roughly 22 mM (69%) exists in the colloidal phase and roughly 10 mM (31%) exists in the soluble phase. Only 2 mM of soluble Ca exist as ionic Ca (Walstra and Jenness, 1984). Ca distribution in milk is 2 to 2.5 times higher in the colloidal phase than in the soluble phase (De la Fuente, 1988, Rajput and Bhavadassan, 1983). The remainder of soluble Ca is associated to citrate, phosphate, and casein monomers. In the

colloidal phase, Ca may interact with phosphoesters, carboxyl groups of casein micelles, or colloidal phosphate and citrate associated with casein micelles (Philippe et al., 2003).

2.6.2. Importance of CCP in Casein Micelle Stability

Approximately 6% (w/w) of dry casein is composed of various minerals, of which calcium phosphate is the largest constituent (Fox and McSweeney, 1998), and it is a key structural element of the casein micelle. CCP, with its positive charge, binds to negatively charged phosphoserine residues and reduces the caseins' negative charge to a level at which hydrophobic interactions dominate (Horne, 1998). CCP may be solubilized at low temperatures and by acidification.

2.6.3. Sodium

Casein micelle stability can be altered by the presence or absence of several key cations, notably calcium, potassium, phosphorus, magnesium, and sodium. The effect of NaCl on properties of casein micelles in both unconcentrated and concentrated milk has been explored in the scientific literature. Addition of 50 mM to 100 mM NaCl to unconcentrated milk reduces the milk's pH, primarily caused by increased dissociation of ion pairs when net ionic strength increases, and the displacement of casein-bound H^+ by Na^+, which results in increasing serum-phase H^+ (van Hooydonk et al., 1986). The addition of NaCl has also been shown to increase micelle hydration, which is thought to increase the heat stability of milk (Creamer, 1985).

The addition of 100 mM to 400 mM NaCl to concentrated bovine milk (18 % and 27 % total solids) is also associated with a number of changes, such as an increase in the amount of ionic calcium and soluble calcium present, and a decrease in zeta potential.

However, no net change in casein micelle size was detected. It was also noted that NaCl addition increased the heat coagulation time of concentrated milk (18% and 27% total solids), and decreased the extent of heat-induced dissociation of micellar κ-CN as determined by SDS-PAGE (Huppertz and Fox, 2006). A decrease in heat-induced dissociation of micellar κ-CN is expected to result in an increase in the heat coagulation time of milk.

2.6.3.1. Sodium's Role in Determination of Milk System Ionic Strength

Milk can be considered a dilute aqueous solution with an ionic strength of roughly 0.073 M. In an ideal solution, ionic strength is defined as a function of the concentration of all ions in solution (Equation 4); however, milk is not an ideal solution and thus activities must be used to calculate ionic strength (Equation 2, Equation 3).

$$a_x \equiv \gamma_x m_x$$

Equation 2: Relationship describing ion activity of substance x, a = activity (mol/L), γ = activity coefficient, m = concentration (mol/L)

$$\gamma = \exp(-0.8 z^2 I^{1/2})$$

Equation 3: Free ion activity coefficient of an ionic species in water at room temperature when ionic strength < 0.1 M, γ = activity coefficient, z = valency of ion i in Equation 4, I = ionic strength of solution.

$$I = \frac{1}{2}\sum_{i=1}^{n} c_i z_i^2$$

Equation 4: Ionic strength of a solution, I = ionic strength c_i = molar concentration of ion i, z_i = charge number of ion i

Addition of NaCl to the milk solution causes an increase in the ionic strength of the milk solution, which in turn causes a decrease in ion activity coefficients (Equation 3). The result is that, in general, the solubility of milk salts increases (Walstra et al., 2006), a phenomenon which some MPC manufacturers may exploit to modify the mineral content of retentate during the ultrafiltration process. In particular, the solubility of calcium phosphate (Equation 5) increases as NaCl is added to milk; increasing *I* results in a decrease in the activity coefficient of Ca^{+2} and HPO_4^{-2}, given that dissociation K_D remains constant (Equation 6).

$$Ca^{+2} + HPO_4^{-2} \leftrightarrow CaHPO_4$$

Equation 5: Chemical equation describing the equilibrium of calcium phosphate in milk

$$K_D = a_{Ca^{+2}} \times a_{HPO_4^{-2}} / a_{CaPO_4}$$

Equation 6: Dissociation constant for calcium phosphate in milk

The ionic strength of the milk solution also plays a strong role in determining the extent of electrostatic repulsion between particles. These particles have negative surface charge; therefore counterions (cations in milk) tend to group near the particle surface, while co-ions (anions in milk) migrate toward the interstitial space between particles. Particle surfaces contain high concentrations of counterions, with the concentration of counterions gradually decreasing as the distance away from the particle surface becomes greater. The accumulated counterions at the particle surface, along with the gradient of decreasing counterion concentration, is known as the electrical double layer. If two

charged particles overlap, their electrical double layers may also overlap, causing the repulsion of the two particles.

However, the addition of NaCl to concentrated milk is known to result in an increase in ionic strength, which would have the effect of reducing the electric shielding parameter 1/k (Equation 7) and collapsing the electrical double layer.

$$1/k \approx 0.30/\sqrt{I}$$

Equation 7:Approximation of the Debye length as provided by Walstra et al.(2006), 1/k = the nominal thickness of the electric double layer, I = ionic strength (mol/L),

Therefore, if the solubility of MPC may be improved by the addition of Na to retentate, depletion of Ca from retentate during the MPC manufacture process, several events may be occurring simultaneously that greatly impact ionic strength, electrostatic interactions, the electrical double layer, and ultimately the interaction free energy between colloidal milk particles. Because the interaction free energy between colloidal milk particles cannot yet be estimated with accuracy in a given system (Walstra et al., 2006), it is critically important that future studies obtain qualitative data on the ion concentrations and activities present within retentate products; if this data is obtained, the role ionic strength plays in protein aggregation upon spray-drying of MPC retentate can be better understood.

2.7. Summary of Literature

Milk protein concentrate (MPC) can be defined as a range of products with varying bovine milk protein content produced from the ultrafiltration of skim milk and

subsequent water removal. The manipulation of processing parameters has allowed manufacturers to create a wide variety of MPC products with varying protein, lactose, fat, and mineral concentrations. Large discrepancies between MPC composition (particularly with respect to key cations) from manufacturer to manufacturer, along with literature demonstrating intent to manipulate mineral content to improve functionality, indicate that (1) manufacturing process and equipment is highly varied, and (2) manufacturers employ different intermediary steps with the intent of replenishing or depleting minerals. Of key interest is solubility of MPC, which has been found to vary widely, despite its importance in dictating the expression of most other functional properties. Prior research has shown a relationship between Na and K content and solubility of MPC, with some evidence that Ca content also plays a role, and that these relationships would be well worth further investigation.

3. Justification, Hypothesis, and Objectives

An extended review of the patent and scientific literature hints at two critical factors that likely influence the solubility of MPC80. The calcium content of the skim milk retentate prior to spray-drying is the first critical factor implicated by several workers in the art (Carr et al., 2002, Dybing et al., 2007), who collectively describe processes for the Ca depletion of retentate by acidification, Na addition, and/or ion exchange, leading to the production of cold-water soluble MPC80 with improved solubility. Additionally, workers in the art of cheese making reference the use of such calcium-depleted MPC in patent literature relating to the manufacture of process cheese (Moran et al., 2001). The second critical factor is the Na content of MPC. Using sodium to fortify either the skim milk starting material or the product obtained by UF/DF has been demonstrated to significantly improve the solubility of MPC.

Despite evidence of the effect of Ca depletion and Na addition on the solubility of MPC, there exists no study in the scientific literature examining the relationship between an MPC manufacture process designed to replace Ca with Na, and proximate composition of retentate intermediates obtained during the manufacture process. Additionally, there are no studies which link manufacturing process of an MPC replenished in Na content, Na and Ca content of retentates and powder, particle size, particle structure, solubility, and changes in hydrophobicity upon powder reconstitution. There is also no mention of an MPC manufacture process that incorporates sodium addition into DF water, which may be a convenient application in industry which prevents the contamination of UF permeate with high Na content.

This thesis project intends to examine the effect of Na addition into DF water on the solubility of MPC. The relationships between MPC composition, solubility, structure, and particle size will be examined. Once these relationships have been elucidated, steps can be taken to control the content of key minerals during the manufacturing process, with the intention of producing more highly soluble MPCs.

MPC can be defined as a product with varying bovine milk protein content produced from the ultrafiltration and subsequent diafiltration of skim milk. Unfortunately, many MPC powders concentrated to 70% protein or above exhibit poor water solubility, and the solubility of these powders varies greatly from manufacturer to manufacturer. This suggests inconsistencies in manufacturing process and parameters. A review of the current scientific literature hints at several critical factors, including powder Na content, that likely influence the solubility of MPC. There exists no study in the scientific literature examining the relationship between MPC processing method, solubility, composition, Ca content, and Na content with particle structure and size distributions both of the dry powder and during reconstitution. The experiment proposed is designed to address the current needs of the scientific literature. Data produced by such a study could be utilized by MPC manufacturers to produce MPCs of consistently high solubility, and by the research community in future work exploring the interactions leading to protein aggregation and insolubility in spray-dried MPC.

The hypotheses of this study were:

1. MPC manufactured utilizing increasing levels of Na in DF water will contain increasing levels of Na and will also show increased solubility upon reconstitution into DI water.
2. MPC manufactured utilizing increasing levels of Na in DF water will exhibit smaller particle sizes, and differences in particle structures during reconstitution will be observed between MPCs manufactured at different treatment levels.

The objectives of this study were:

1. To develop a method of replenishing MPC retentate of Na content by adding NaCl into DF water, and to show evidence that this method yields an MPC with improved solubility upon reconstitution into water.
2. To show that, at increasing levels of NaCl addition into DF water, progressively more Na is present in retentate and final powder, while decreasing levels of Ca are present in retentate and final powder.
3. To show evidence of changes in particle size and structure that exist between Na-replenished MPC and MPC that has not been replenished with Na upon reconstitution into water.

4. MATERIALS AND METHODS

4.1. Experimental Design

The model of the randomized complete block is:

$$y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

Definitions of terms are as follows:

τ_i = main effect of the *i*th sodium chloride treatment level

β_j = main effect of *j*th block

ε_{ij} = random error term

i = 1, 2, 3, 4

1 = 0 mM (0 %)

2 = 50 mM (0.29 %)

3 = 100 mM (0.58 %)

4 = 150 mM (0.88 %)

j = 1, 2, 3

1 = batch 1

2 = batch 2

3 = batch 3

Data from previous pilot plant trials was used to approximate statistical power. Three preliminary trials were performed using the manufacture method described in section 4.2; one trial utilized NaCl treatment level 1, one trial utilized NaCl treatment level 2, and one trial utilized NaCl treatment level 3. From these preliminary trials, it was determined that only one treatment level could be reliably tested per day, and that one

batch of skim milk should be utilized to produce each of the four treatments (to attempt to minimize any batch effect). This dictated the incorporation of a blocking factor into the experiment design. These three preliminary trials indicated approximately 10.0% difference in solubility between the most effective treatment level and control samples. Additionally, it was observed that the standard deviation for MPC solubility was approximately 2.6 %.

Statistical power is defined as the probability that a test with the specified assumptions will correctly reject the null hypothesis when the alternate hypothesis is true, and is defined by the series of equations in Appendix A.

Using Minitab v15.1 it was calculated that, with four treatment levels, 88.0 % power was obtained by completing each treatment three times (Figure 10). This indicated that the experiment had an 88.0 % chance of detecting a 10 % difference in solubility if it truly exists. The actual order of trials performed in this experiment is also shown (Table 6).

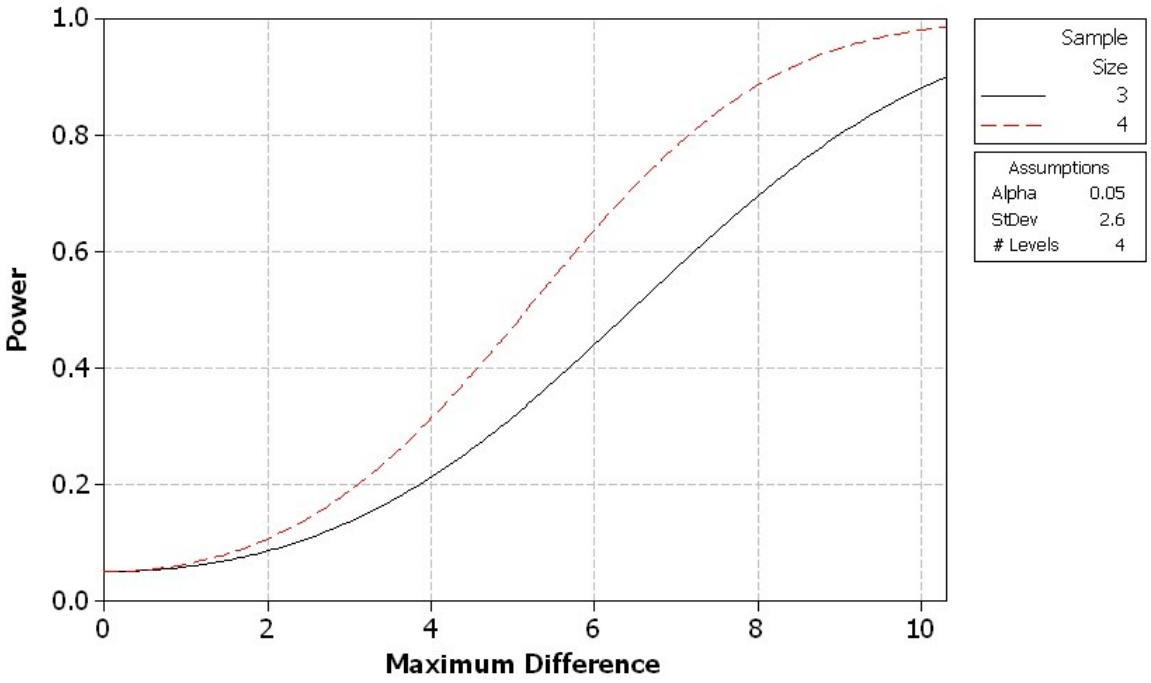

Figure 10: Power curve for one-way ANOVA for MPC manufacture. x axis is maximum detectable difference in % solubility

Table 6: Actual order of MPC trials performed

Block	Day	Treatment (% NaCl in DF H_2O)	Treatment Level
1	1	0.00	1
1	2	0.29	2
1	3	0.58	3
1	4	0.88	4
2	5	0.58	3
2	6	0.88	4
2	7	0.29	2
2	8	0.00	1
3	9	0.88	4
3	10	0.00	1
3	11	0.29	2
3	12	0.58	3

4.2. MPC Manufacture

Milk was stored, collected, and distributed for MPC manufacture trials according to Figure 11. Three batches of 560 kg of pasteurized skim milk were obtained from Producer's Dairy Foods, Inc. (Fresno, CA) and stored at 4 °C. One batch was utilized per block, and milk was stored a maximum of one week before use. One block consisted of four trials (one trial at each of the four treatment levels), and one trial was conducted per day. One trial consisted of 140 kg skim milk being transferred to the retentate holding tank attached to an R12 Pilot Plant (Niro Inc, Hudson, WI) (Figure 11) cross-flow unit with dual 10 KDa cut-off (nominal) spiral-wound polyether sulfone (PES) membranes (model MT3B-3838, approximate length = 1.2 m, Snyder Filtration, Vacaville, CA).

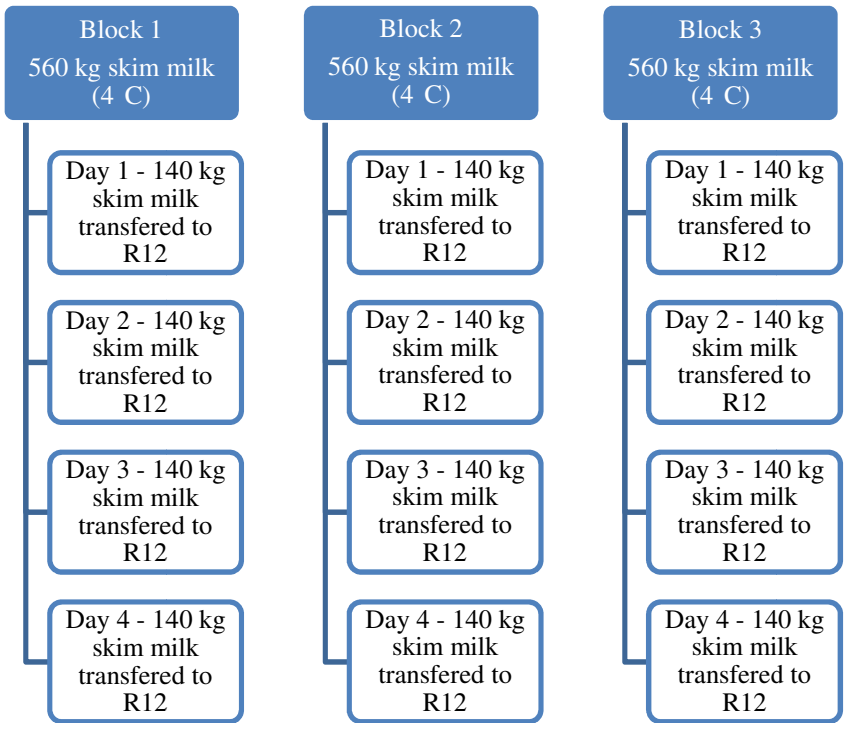

Figure 11: Storage and distribution of skim milk for MPC manufacture trials

Prior to each day's trial, the R12 equipment was flushed with soft H_2O for 30 min, then shut down and allowed to empty completely. Once one day's skim milk was transferred to the R12 as per Figure 11 , the UF and DF manufacture process was conduced according to Figure 12. UF commenced at 5.9 °C ± 1.2 °C. During UF, temperature was allowed to increase such that, by the end of the UF process, the temperature was 19.7 °C ± 1.5 °C. DF H_2O was prepared by transferring 117 kg soft H_2O (GE SmartWater, Fairfield, CT) to a jacketed kettle (60 gallon capacity, serial number 50347, Will-Flow Corp., Charlevoix, Michigan) with an overhead mixer (P/N 123856, Form Factor 1.33, Lightnin, Rochester, NY) holding at 26.7 °C, and mixing for 15 min after addition of non-iodized NaCl (Evaporated Food Grade Salt, United Salt Corp., Houston, TX). Each UF and DF step was monitored by weighing the permeate and proceeding when permeate removal weight equaled 117 kg (representing a retentate concentration factor of 4.2). Temperature and pH were recorded during the manufacture process. One trial was conducted per day. At the end of each trial, membranes were cleaned by washing with soft H_2O for 30 min at 35 °C, caustic solution (Chlorinated Mechanical and CIP Cleaner for Protein Soils) (Ecolab, St. Paul, MN) for 1 h at 49 °C, soft H_2O for 30 min at 35 °C, acidic solution (HD PL-10 Plus Acid Detergent for Pipeline and Bulk Tank Equipment) (Ecolab, St. Paul, MN) for 1 h at 48 °C, followed by soft H_2O for 30 min at 35 °C, in preparation for the next day's trial.

Immediately after the DF3 process was completed, retentate was collected, weighed, and spray-dried using a Niro Filterlab (Hudson, WI) with consistent inlet feed. Inlet temperature was 208.7 ± 19.7 °C. Outlet temperature was 82.0 °C ± 0.8 °C. The powder was collected, weighed, and split into two batches; one batch was stored at 22 °C

± 3 °C and was used for all forthcoming described experiments, and the other batch was stored at 4 °C in plastic airtight containers for later analysis.

During the manufacture, samples (200 ml) of skim milk, as well as products obtained by UF and DF, were withdrawn and stored for later analysis. Skim milk was withdrawn after loading skim milk into the holding tank of the R-12 Pilot Plant and the skim milk in the holding tank had been mixed thoroughly (prior to initiation of the UF process). Products of UF and DF were sampled by collecting the continuously flowing product out of the retentate return as it returned to the retentate holding tank, immediately after the required amount of permeate had been removed. All samples were preserved by adding 0.02 % NaN_3 (EMD Chemicals, Gibbstown, NJ) and storing at 4 °C.

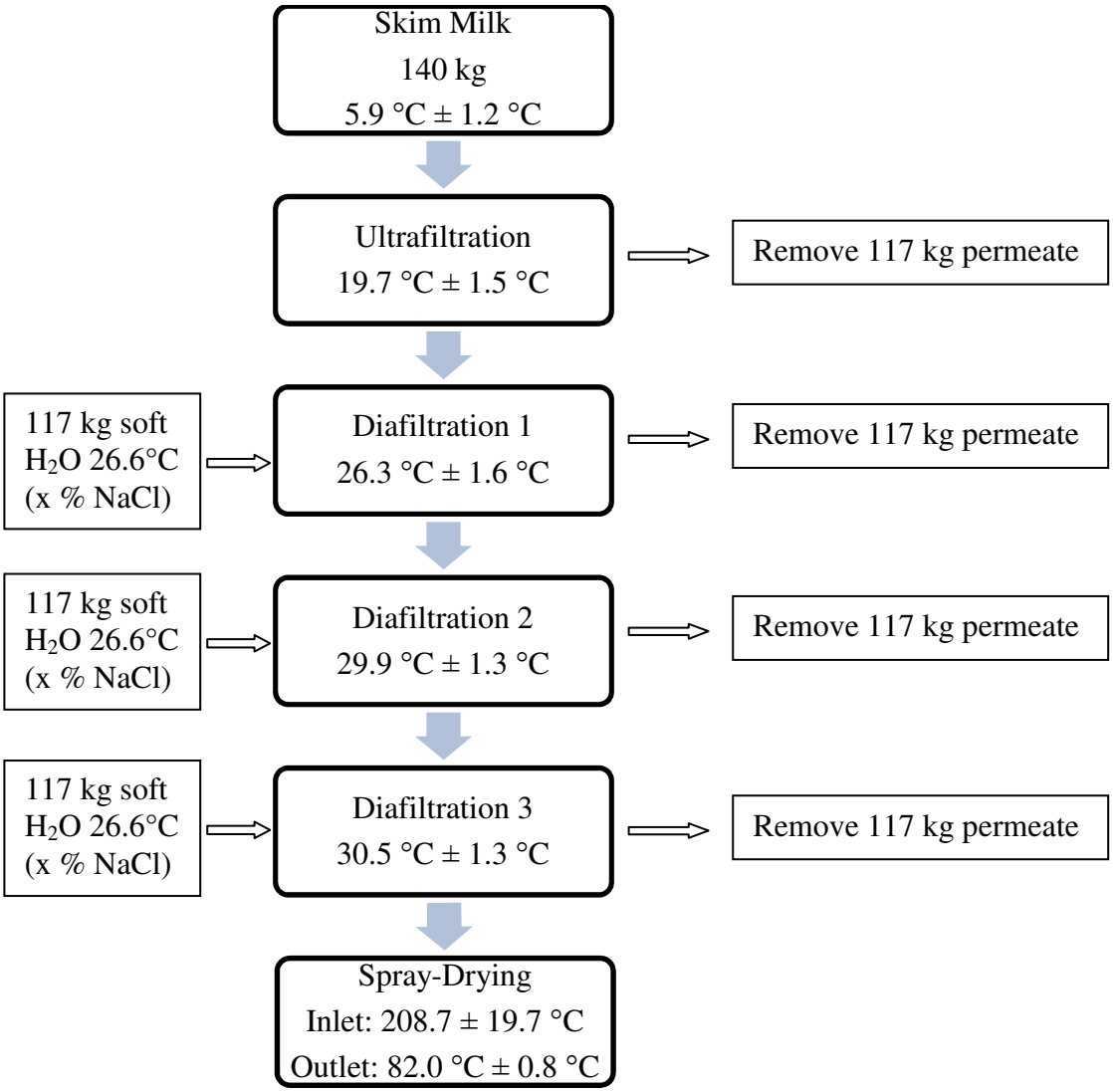

Figure 12: Schematic of a single MPC manufacture trial; x = no NaCl (treatment level 1), 0.29 % NaCl (50 mM) (treatment level 2), 0.58 % NaCl (100 mM) (treatment level 3), or 0.88 % NaCl (150 mM) (treatment level 4) in DF H_2O. Retentate (DF3) yields varied between 11 and 13 kg; dry MPC yields varied between 0.9 kg and 1.3 kg.

4.3. Solubility Analysis

4.3.1. Laboratory Stage Mixer

Solutions of MPC (5% w/w) were made by placing an octagonal magnetic stir bar (8 x 38mm) in a 250 ml beaker, weighing 95.0 g DI H2O in the beaker, placing 5.0 g MPC in a weigh dish, transferring the MPC to the DI H2O, and hand-mixing (100 clockwise revolutions with a thin spatula), prior to reconstitution using a laboratory stage mixer (R010 Power, IKA Works, Wilmington, NC). Each was allowed to reconstitute at 960 rpm (speed setting #8) at 23 °C ± 1.0 °C for 1 h and 3 h. One complete block was tested at a time, and two replicates of each treatment were tested at a time. Aliquots (13 ml) of these samples were transferred to a series of 15 ml falcon tubes and centrifuged at 700 x g for 10 min at 23 °C. The supernatant was separated from the pelleted material by withdrawing supernatant into a pipette. Percent solubility was calculated as supernatant total solids (TS) divided by bulk solution TS, multiplied by 100. TS was determined using CEM LabWave 9000 (CEM Corp, Matthews, NC) using parameters of 100% power and 4 min drying time, after running duplicate samples under vacuum oven drying (AOAC 927.05) to verify accuracy of LabWave 9000 measurements. Measurements were conducted in duplicate. A diagram of this experiment is shown in

Figure 13.

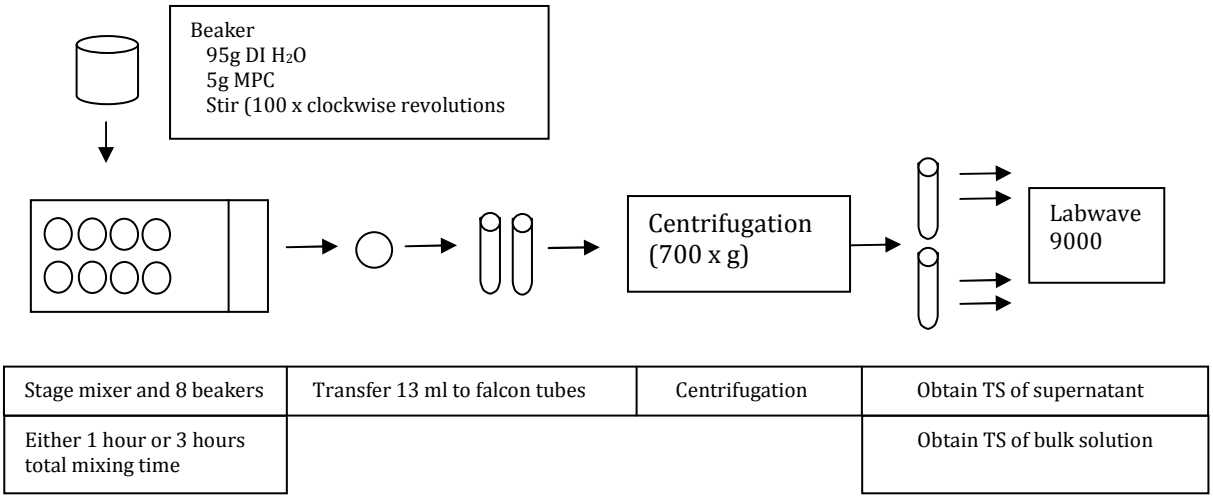

Figure 13: Diagram of laboratory stage mixer solubility experiment

4.3.2. Insolubility Index

One-hundred ml of water (24 ˚C ± 0.2 ˚C) was poured into the mixing jar (bottom diameter = 7.11 cm, blade diameter = 5.08 cm) (Waring Commercial Blender [Model 34B197, 120 Volts AC, 50 Hz to 60 Hz, 7.0 Amps], Torrington, CT). An aliquot of MPC (5 g) was placed into the mixing jar. The analysis then proceeded according to Insolubility Index: GEA Niro analytical method A 3 a (Anonymous, 2010c). Three drops of Antifoam B Emulsion (Sigma-Aldrich, St. Louis, MO) were added, and mixing commenced at 3800 rpm for 90 sec. The solution was allowed to sit for 15 min. After 15 min. elapsed, the solution was stirred with a thin spatula and transferred to two 50 ml centrifuge tubes. Centrifugation took place at 910 rpm for 5 min. A pipette was used to dispose of all sediment-free liquid more than 5 ml above the sediment layer. The centrifuge tubes were then filled with DI water to the 50 ml mark, the sediment was dispersed with a thin spatula, and centrifugation again took place at 910 rpm for 5 min.

The amount of sediment remaining was reported in ml. Measurements were conducted in duplicate.

4.3.3. Solubility Using Modified Method of Anema et al. (2006)

Solutions of MPC (5% w/w) were made in a 400 ml beaker by weighing 95.0 g DI H_2O in the beaker, placing 5.0 g MPC in a weigh dish, transferring the MPC to the DI H_2O, and hand-mixing (100 clockwise revolutions with a thin spatula). This solution was placed into a 30 °C water bath. Mixing immediately commenced at 600 rpm (speed setting #5) using a propeller-blade (blade diameter = 4.4 cm) attached to an overhead stirrer (StedFast Stirrer [Model SL600], Fisher Scientific, Tustin, CA). After 30 min., samples of MPC solutions were then withdrawn, and the supernatant was obtained by centrifugation at 700 x *g* for 10 min. Percent solubility was calculated as supernatant total solids TS divided by bulk solution TS, multiplied by 100. TS was determined using CEM LabWave 9000 (CEM Corp, Matthews, NC) using parameters of 100% power and 4 min. drying time. Measurements were conducted in duplicate.

4.3.4. Solubility in pH-adjusted Environments

Solutions of MPC (5% w/w) were made by placing an octagonal magnetic stir bar (8 x 38mm) in a 250 ml beaker, weighing 95.0 g DI H_2O in the beaker, placing 5.0 g MPC in a weigh dish, transferring the MPC to the DI H_2O, and hand-mixing (100 clockwise revolutions with a thin spatula), prior to reconstitution using a laboratory stage mixer (R010 Power, IKA Works, Wilmington, NC). Each was allowed to reconstitute at 960 rpm for 10 min. to allow for dispersion of material. pH of all samples were recorded. While mixing, 1% NaOH solution was used to adjust pH of samples treatment levels 1

through 3 to pH of treatment level 4. Samples were allowed to mix at 23 °C ± 1.0 °C until 3 h total mixing time was achieved. Samples were checked at 30 min. intervals to ensure consistency in pH, and readjusted with 1% NaOH if necessary. Aliquots (13 ml) of these samples were transferred to a series of falcon tubes and centrifuged at 700 x *g* for 10 min at 23 °C. The supernatant was separated from the pelleted material by withdrawing supernatant into a pipette and transferring to a 15 ml falcon tube. Percent solubility was calculated as supernatant TS divided by bulk solution TS, multiplied by 100. TS was determined using CEM LabWave 9000 (CEM Corp, Matthews, NC) using parameters of 100% power and 4 min. drying time. Measurements were conducted in duplicate. Figure 14 diagrams this experiment.

In a separate experiment, solutions of MPC (5% w/w) were made by placing an octagonal magnetic stir bar (8 x 38mm) in a 250 ml beaker, weighing 95.0 g DI H_2O in the beaker, placing 5.0 g MPC in a weigh dish, transferring the MPC to the DI H_2O, and hand-mixing (100 clockwise revolutions with a thin spatula), prior to reconstitution using a laboratory stage mixer (R010 Power, IKA Works, Wilmington, NC). Each was allowed to reconstitute at 960 rpm for 10 min. to allow for dispersion of material. pH of all samples were recorded. While mixing, 1% HCl solution was used to adjust pH of samples treatment levels 2 through 4 to pH of treatment level 1. Samples were allowed to mix at 23 °C ± 1.0 °C until 3 h total mixing time was achieved. Samples were checked at 30 min. intervals to ensure consistency in pH, and readjusted with 1% HCl if necessary. Aliquots (13 ml) of these samples were transferred to a series of falcon tubes and centrifuged at 700 x *g* for 10 min at 23 °C. The supernatant was separated from the pelleted material by withdrawing supernatant into a pipette. Percent solubility was

calculated as supernatant total solids TS divided by bulk solution TS, multiplied by 100. TS was determined using CEM LabWave 9000 (CEM Corp, Matthews, NC) using parameters of 100% power and 4 min drying time. Measurements were conducted in duplicate. Figure 14 diagrams this experiment.

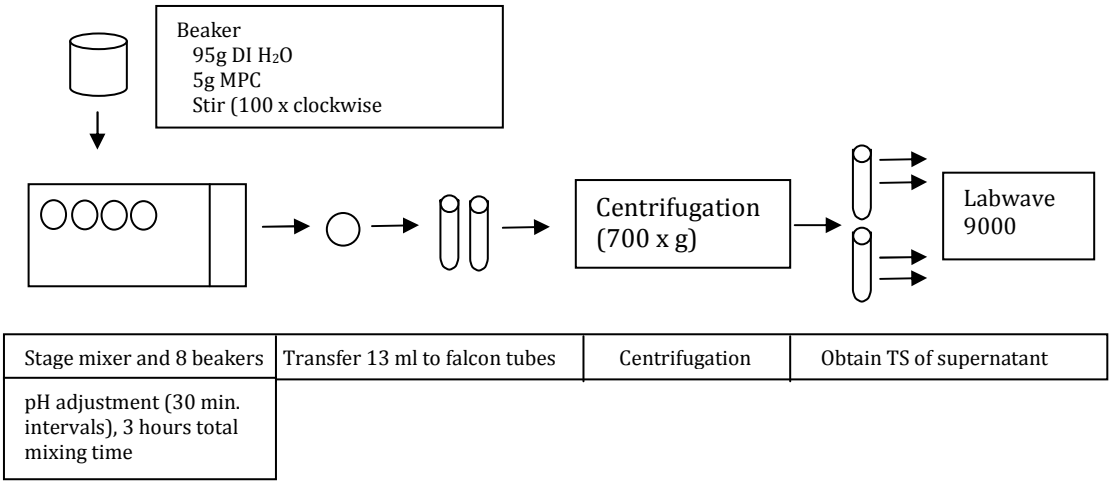

Figure 14: Solubility of experiment testing solubility in pH-adjusted environments

4.4. Chemical Composition of MPCs Manufactured by Na Addition to DF H_2O

4.4.1. FTIR Analysis

The protein, casein, lactose, fat, and total solids content of skim milks and products obtained by UF and DF was analyzed with Milkoscan FT2 (Foss, Eden Prairie, MN) utilizing Foss Integrator software package version 1.5.0 (Foss, Eden Prairie, MN). Measurements were conducted in duplicate. Prior to run, instrument was zeroed with Winescan™/Grapescan™ Zero Liquid Salt (Foss Analytical A/S, Eden Prairie, MN). Instrument was soaked with Foss-Soak (Foss Analytical A/S, Eden Prairie, MN) containing enzyme, surfactant, and sodium carbonate. Instrument was cleaned with S-470

Cleaning Agent (Foss Analytical A/S. Eden Prairie, MN). Instrument was calibrated with DQCI Services: Dairy Quality Control California Milk Standards (product #110300) (DQCI, Mounds View, MN).

4.4.2. Nitrogen Analysis

The protein content of skim milks, products obtained by UF and DF, and MPCs were analyzed using Elementar rapid N cube (Elementar Analysensysteme GmbH, Hanau, Germany). Prior to sample run, instrument was blanked three times with O_2 and prepared for calibration with two L-aspartic acid (Sigma-Aldrich, St. Louis, MO) samples weighing 100 mg ± 10 mg each, utilizing the "RunIn" method. Instrument was calibrated using "aspartic acid" method, using three L-aspartic acid samples weighing 100 mg ± 10 mg each. Daily correction factor was checked to be between 0.95 and 1.05 before proceeding; if daily correction factor was lower or higher, then blanking and calibration were repeated until daily correction factor was between 0.95 and 1.05. Volume of tin capsule used for sample delivery of skim milk, UF, and DF products was 4.75 mm * 11 mm (Elementar Americas, Mt Laurel, NJ). Weight of sample delivery was 100 mg ± 10 mg, utilizing "100 mg" delivery method. Tin capsule with sample was flushed with Ultra High Purity (UHP) O_2 (Airgas West, San Luis Obispo, CA) for 3 sec. prior to closure of tin capsule. Sample delivery of dry MPCs was conducted by utilizing Elementar-supplied tin foil dimension 50 mm * 50 mm (Elementar Americas, Mt Laurel, NJ). Weight of sample delivery was 100 mg ± 10 mg, utilizing "100 mg" method. Protein was calculated using protein correction factor 6.38, by rapid N Software v.3.2.6 (Elementar Analysensysteme GmbH, Hanau, Germany) according to the Dumas method (AOAC 993.13). Measurements were conducted in duplicate.

4.4.3. Minerals Analysis

The concentration of Ca, Na, K, and Mg in skim milks and products obtained by UF and DF was determined by the Institute for Integrated Research on Materials, Environment and Society (IIRMES) (Long Beach, CA). Samples (10 ml) were transferred to a series of 15 ml falcon tubes, assigned a 5-digit code at random, and shipped overnight from California Polytechnic State University San Luis Obispo to IIRMES. Samples were held at 4 °C during transit. Upon receiving, samples were frozen and stored for later analysis.

Prior to minerals analysis, samples were removed from frozen storage and placed in a 50 °C water bath for 30 min to return sample flowability. Microwave-assisted acid digestion was utilized to obtain complete sample digestion, followed by sonication and heating at 65 °C for 2 h. 100 µl of digested, heated, and sonicated sample was placed in a 15 ml conical vial, along with 200 µl Rh/Tm solution. Minerals analysis was conduced using Hewlett Packard 4500 ICP-MS (Agilent Technologies, Santa Clara, CA) according to EPA 200.8m; the concentration of Ca, Na, K, and Mg in MPCs was analyzed according to EPA 6020m. Instrument Calibration Standard 2 Claritas PPT® from Spex Certiprep (Metuchen, NJ) was used to generate a standard response curve for Ca, Na, Mg, and K. Certified Reference Material (CRM) (cat. #018) from Environmental Resource Associates (ERA) (Arvada, CO) was used to generate a standard response curve for Ca. CRM (cat. #025) from ERA was used to generate a standard response curve for Mg. Measurements were conducted in triplicate.

4.5. Physical Properties of MPCs Manufactured by Na Addition to DF Water

4.5.1. Particle Size Diameter Distribution Analysis of MPC

Particle size diameter distribution of dry MPC was determined using Coulter LS 230 with Dry Powder Module (Beckman Coulter, Brea, CA). The instrument was allowed to adjust for electrical offsets and align the laser prior to measuring background. Background was measured for 60 sec. Sample loading was measured for 60 sec. Obscuration was held between 4% and 7% during the runtime by adjusting speed of the auger attached to the feed mechanism. Voltage measurements from the detectors were converted to particle size diameter distributions by Beckman Coulter LS Software v.3.29 August 2003 (Brea, CA) utilizing the Fraunhofer model of light scattering. A complete particle size distribution from 0.4 µm to 2000 µm, for each powder, was obtained in triplicate.

4.5.2. Particle Size Distribution Analysis of MPC During Reconstitution

Solutions of MPC (5% w/w) were made by placing an octagonal magnetic stir bar (8 x 38mm) in a 250 ml beaker, weighing 95.0 g DI H_2O in the beaker, placing 5.0 g MPC in a weigh dish, transferring the MPC to the DI H_2O, and hand-mixing (100 clockwise revolutions with a thin spatula), prior to reconstitution using a laboratory stage mixer (R010 Power, IKA Works, Wilmington, NC). Each was allowed to reconstitute at 960 rpm at 23 °C ± 1.0 °C for 3 h, prior to particle size diameter distribution measurement using Coulter LS230 with Fluid Module (Beckman Coulter, Brea, CA). At initial start-up, and thereafter once per hour, the instrument was allowed to adjust for electrical offsets and align the laser prior to measuring background. Background was

measured for 60 sec. Sample loading was measured for 60 sec. Sample was loaded until obscuration was between 10 % and 12 %. Pump speed was set to 51 (medium speed), and sonication was not used. Voltage measurements from the detectors were converted to particle size diameter distributions by Beckman Coulter LS Software v.3.29 August 2003 (Beckman Coulter, Brea, CA) utilizing the Fraunhofer model of light scattering. A complete particle size distribution from 0.4 µm to 2000 µm, for each sample, was obtained in triplicate. The fluid module was flushed with water for 15 min. between each sample.

4.5.3. Confocal Laser Scanning Microscopy (CLSM).

An Olympus FV1000 confocal laser scanning biological microscope (Olympus America Inc., Center Valley, PA, USA) was used to examine the structure of MPC protein aggregates dissolved in solution, and possible changes in lipid surface coverage (Fillery-Travis et al., 2000) of protein aggregates occurring with treatment level. In a 50 ml falcon tube, 1 g powder was weighed to the nearest 0.0001 g. 30 ml DI H_2O was added, and the solution was vortexed 1 min. to mix. 100 ul was transferred to a microfuge tube. To the microfuge tube, 2 µl of Nile Red (NR), (9-diethylamino-5H-benzo[R]phenoxazine-5-one) dye (1 mg/ml in acetone) was added, and the tube was vortexed to mix. Then, 5 µl Fast Green (FG) (disodium2-[[4-[ethyl-[(3-sulfonatophenyl)methyl]-amino]phenyl]-[4-[ethyl-[(3-sulfonatophenyl)methyl]azaniumylidene]-cyclohexa-2,5-dien-1-ylidene]methyl]-5-hydroxybenzenesulfonate) dye (1 mg/ml in nanopure H_2O) was added to the microfuge tube, and the tube was vortexed to mix. 25 µl of the sample + dye mixture was added to a microscope slide. Quickly, 50 µl 0.5 % warm agarose solution was added to the slide and

mixed gently using the micropipette tip. A UPLAPO20x objective lens and UPLFL60XO oil-immersion objective lens were used. NR excitation was performed with the 543nm HeNeG laser. FG excitation was performed with the 633nm HeNeR laser. A 3D image was obtained by setting a defined z-section prior to scanning the sample.

4.5.4. Statistical Analyses

The statistical analyses of solubility on the laboratory stage mixer for both one hour and three hour reconstitution time were conducted using the GLM command in Minitab (v.16.1, Minitab Inc., State College, Pennsylvania). Block was a random effect. Treatment was a fixed effect. All statistical tests were performed at a significance level of $\alpha = 0.01$ in order to compensate for the large number of tests throughout the analysis. Initial models were run to check for a statistically significant ($\alpha = 0.01$) interaction; if no statistically significant interaction was found, the interaction term was dropped and the model was rerun. Tukey's method was used to compare treatment means. In addition histograms of residuals, normal plot of residuals, plots of residuals versus fits, and plots of residuals versus order were used to test model adequacy. Lavene's test was used to check for unequal error variance between treatments. The statistical analysis of ISI was conducted using the GLM command in Minitab (v.16.1) as described above. The statistical analysis of solubility according to the method of Anema et al. (2006) was conducted using the GLM command in Minitab (v.16.1) as described above. The statistical analysis of solubility in pH adjusted environments was conducted using the GLM command in Minitab (v.16.1) as described above. The statistical analysis of protein content of dry MPC, as determined by Elementar rapid N, was conducted using the GLM command in Minitab (v.16.1) as described above. The statistical analysis of moisture

content of dry MPC was conducted using the GLM command in Minitab (v16.1) as described above. The statistical analysis of MPC particle size upon reconstitution was first conducted by analyzing the d90 and mean as described above. Then, d_{90} was subtracted from d_{10} to create a new variable (d_{80}), treating NaCl as a quantitative variable, and using the GLM command in Minitab (v.16.1) as described above. The statistical analysis of the right tail of MPC particle size distribution upon reconstitution was analyzed by the following method: the d_{50} was subtracted from the d_{90} to create a new response variable, and the GLM command in Minitab (v16.1) was used, treating NaCl as a quantitative variable.

The regression analyses for solubility on the laboratory stage mixer for both one hour and three hour reconstitution time were performed using the General Regression command in Minitab (v16.1); NaCl concentration was the predictor. The regression analysis for solubility according to the method of Anema et al. (2006) and ISI were performed using the General Regression command in Minitab (v16.1); the mean-centered NaCl concentration was the predictor to reduce multicollinearity.

The statistical analysis of Ca, Na, K, and Mg in skim milks and products obtained by UF and DF, on both wet basis and total solids basis, was conducted using SAS (v.9.1, SAS Institute, North Carolina). The difference in Ca, Mg, K, and Na content at DF1, DF2, and DF3 process levels from UF process levels, on wet basis, was analyzed using distinct PROC MIXED statements, with process as a random effect, utilizing autoregressive model 1 (ar1) variance-covariance structure for Ca, Mg, and K analysis, and unstructured variance-covariance structure for Na. LSMEANS and Tukey's comparisons in all PROC MIXED statements were used to aid in analysis interpretation.

On total solids basis, the difference in Ca, Mg, K, and Na content at DF1, DF2, and DF3 process levels from UF process levels was analyzed using distinct PROC MIXED statements, with process as a random effect, utilizing SAS (v.9.1), and variance-covariance structures were as follows: (ar1) for Ca and Mg, and variance components for Na and K. A statistical analysis comparing the FOSS and FTIR obtained protein data was conducted using SAS (v.9.1). The statistical analysis of the ratio of Ca to Mg in the DF3 product on dry basis was conducted using the general regression command in Minitab (v16.1); the mean-centered NaCl concentration was the predictor to reduce multicollinearity.

5. RESULTS AND DISCUSSION

5.1. Temperature and pH of products obtained by MPC manufacture

MPC was manufactured according to the method outlined in section 4.1. It is known that the temperature of UF and DF processes can affect the mineral distribution of retentates manufactured by UF and DF retentates. The pH and temperature of each material obtained after the completion of each step in the MPC manufacture process, by block, treatment, and sample, are displayed in Appendix B, along with the accompanying statistical analyses. Because it is known that temperature and pH affect several important qualities of retentate, including the ratio of bound Ca to ionic Ca and retentate viscosity, that may have an impact on the solubility of the final powder, it was imperative to record the temperature of the skim milk, as well as that of the products obtained by the various UF and DF steps during manufacture. The changes in pH and temperature are summarized by Figure 16 and Figure 17.

At $\alpha = 0.01$, there was a statistically significant difference ($p < 0.001$) in pH due to process level, and there was also a statistically significant ($p = 0.002$) interaction between process and treatment. The MPC manufacture process, particularly diafiltration, is known to deplete soluble minerals from the retentate, including the hydrogen ion which directly contributes to the pH of the system. The general trend towards basic, rather than acidic, retentates is consistent with what is expected during the DF processes. As hydrogen ions are removed, the pH should increase according to the equation (Figure 15). In contrast, the downward trend observed in the pH of the UF process is likely due to two factors. The first is that mean temperature increased from SM to UF, which may drop pH; the second is that the UF process by itself is not known to efficiently remove soluble

minerals. A much higher degree of mineral removal has been observed during the DF processing steps. If this is the case, then a progressive concentration of hydrogen ions during UF may occur, leading to a drop in pH.

$$pH = -\log(H^+)$$

Figure 15: pH, defined as the negative log of hydrogen ion activity in a solution

As determined by the test of effect slices, there was also a statistically significant ($p < 0.001$) difference in pH within the DF3 process. This may be attributable to differences in the final concentration factor and protein content of the retentate, though this was not explicitly tested in the analysis.

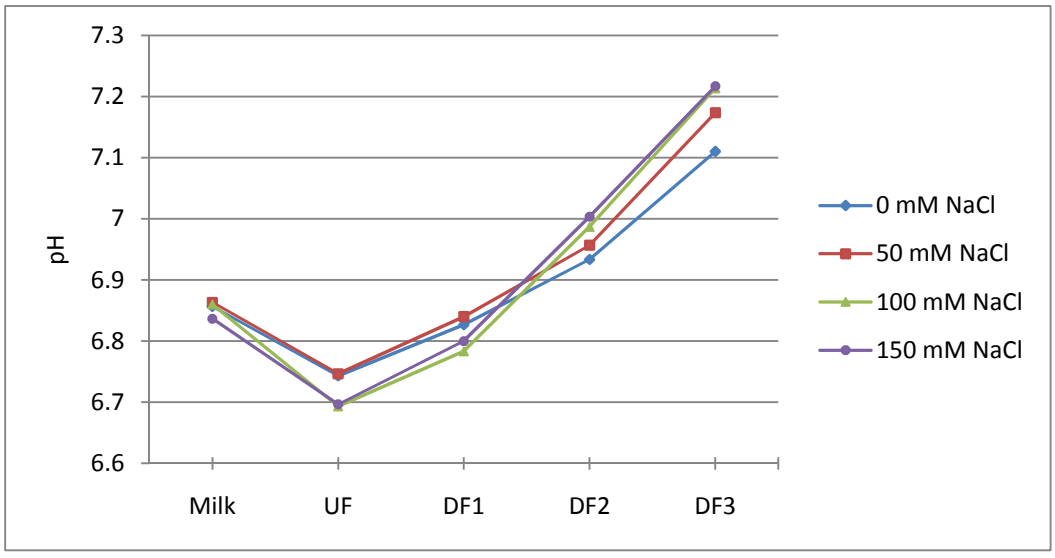

Figure 16: Mean pH (unadjusted for temperature) of products obtained as the result of the UF and DF manufacturing processes, by NaCl treatment level

At $\alpha = 0.01$ there was a statistically significant ($p < 0.001$) difference in temperature due to process, but no statistically significant difference due to treatment ($p = 0.138$). Tests of effect slices indicated that mean temperature was not statistically significantly different within each process level.

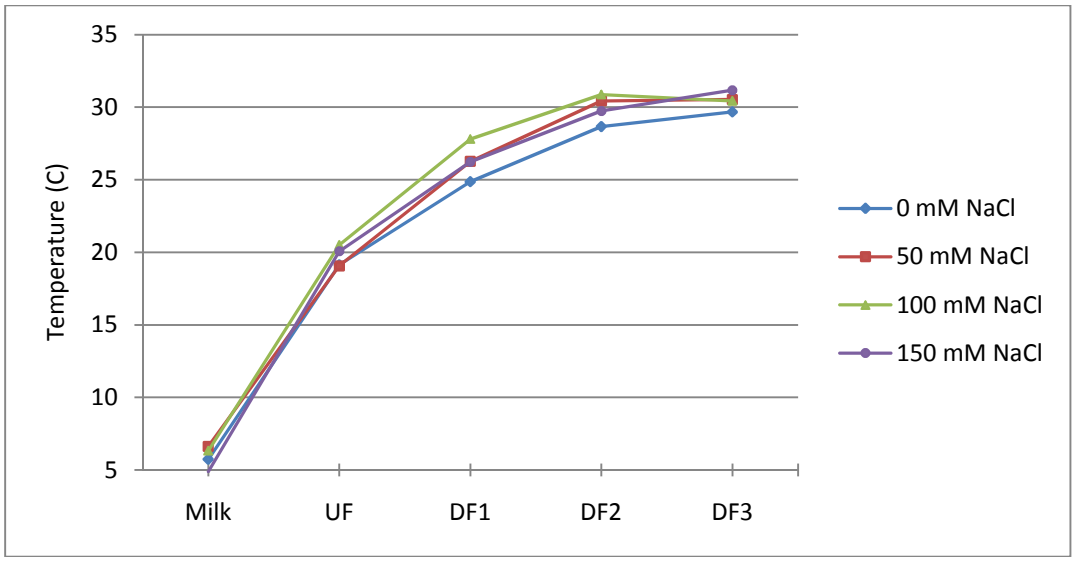

Figure 17: Mean temperature (°C) of products obtained as the result of the UF and DF manufacturing processes

The lack of statistically significant effect slices (save for pH within the DF3 slice as mentioned above) suggests that the manufacture process took place in a consistent fashion with respect to pH and temperature. Though it is clear that both pH and temperature changed throughout the manufacture as a whole, the lack of statistically significant effect slices indicates that the process was consistent across all treatment levels, and thus any observed differences in samples cannot be easily attributed to pH or temperature of manufacture. Treatment level, then, is implicated as the driver of any differences observed between samples.

5.2. Solubility of Manufactured MPCs

5.2.1. One Hour Reconstitution

The data collected according the method outlined in section 4.3.1, pertaining to one hour reconstitution time, is shown in Appendix C. The statistical analysis of solubility on the laboratory stage mixer for one hour reconstitution time was conducted using the GLM command in Minitab according to Section 4.5. The interaction between block and treatment was not statistically significant at $\alpha = 0.01$, and thus the model was rerun without the interaction term. There was a statistically significant difference in mean solubility due to the effect of NaCl treatment ($p < 0.001$). Tukey's simultaneous comparisons state that each treatment level was statistically significantly different from each other (Figure 18).

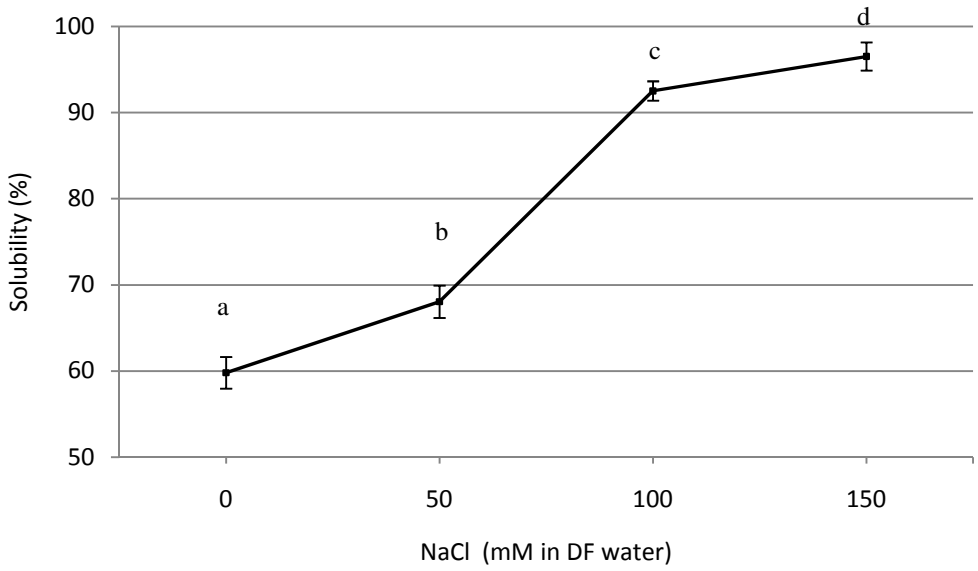

Figure 18: Mean solubility after one hour on laboratory stage mixer, results averaged across all blocks and replicates; different superscripts denote solubility results that are statistically significantly different from each other according to Tukey's simultaneous tests (Appendix C)

These results indicate that the addition of NaCl during DF steps significantly increased the solubility of the manufactured MPC when it was reconstituted for one hour. With 99 % confidence, the addition of 50 mM NaCl into DF water significantly increased ($p < 0.001$) the mean solubility of MPC by between 4.53 % and 11.97 %. The addition of 100 mM NaCl significantly increased ($p < 0.001$) the solubility by between 29.00 % and 36.44 %. The addition of 150 mM NaCl significantly increased ($p = 0.005$) the solubility by between 33.00 % and 40.44 %.

The largest increase in solubility at 1h mixing time was observed between MPC manufactured 50 mM NaCl in DF water and MPC manufactured at 100 mM NaCl in DF water. Therefore, it is recommended that, if a food manufacturer desires to mix MPC for one hour at 22 °C, MPC manufactured utilizing at least 100 mM NaCl in DF water would have significant advantages over those manufactured at lower levels tested. If the

absolute highest solubility is desired, then a significant increase in solubility can be obtained by moving to an MPC manufacture process that incorporates 150 mM NaCl in DF water.

A regression analysis was performed to further elucidate the connection between NaCl addition to DF water and the resulting increase in solubility. The tests associated with this analysis are located in Appendix C. With 99% confidence, the addition of 1 mM NaCl to DF water resulted in an increase in solubility between 0.23% and 0.31%. The relationship between MPC solubility at 1 hour and NaCl concentration in DF water is described by the following equations. The equation for block 1 is: solubility_1hr = 78.6238 + 0.26925(conc-75). The equation for block 2 is: solubility solubility_1hr = 79.01 + 0.26925(conc-75). The equation for block 3 is: solubility_1hr = 80.05 + 0.26925(conc-75). A scatterplot of solubility vs. NaCl concentration is shown (Figure 19). It must be noted that predictions at or near 150 mM may be above 100 %. Therefore, this regression model should be used for descriptive purposes, and not for prediction at the highest levels.

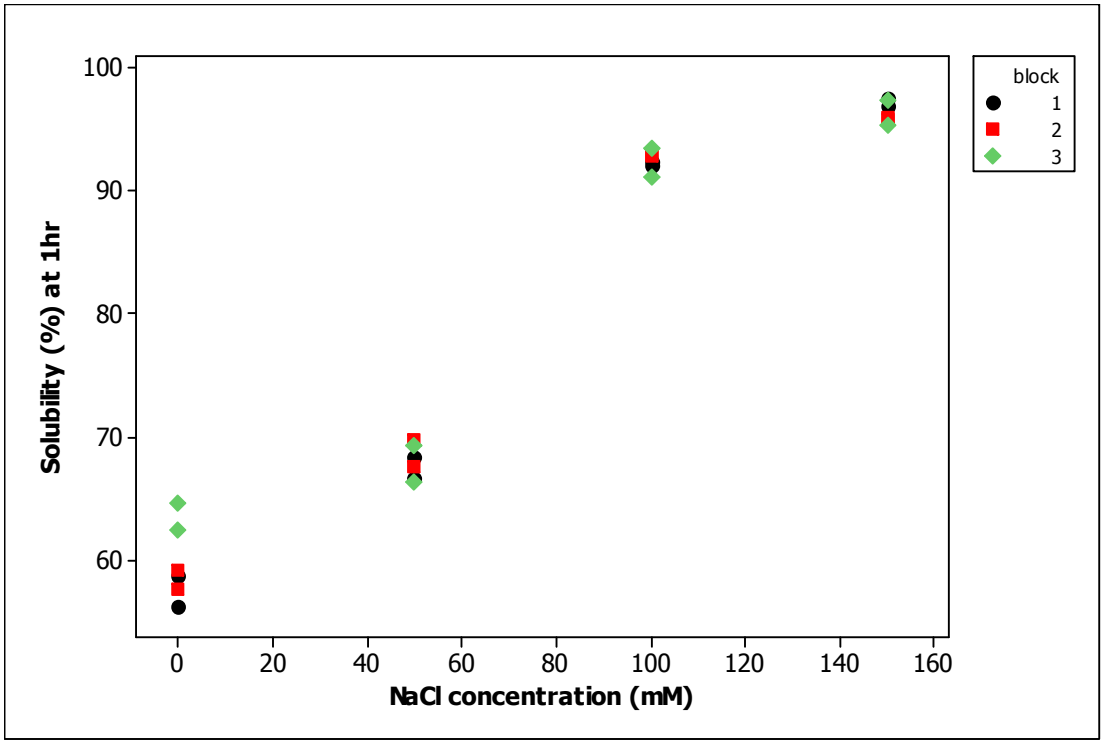

Figure 19: Scatterplot for solubility vs NaCl concentration (mM) at 1h

That data above is consistent with observations that sodium addition to either skim milk or retentate obtained by UF/DF processes may yield a material which, upon spray-drying, exhibits a higher water-solubility than such spray-dried material that has not undergone such sodium addition (Carr et al., 2002, Moran et al., 2001). However, retentate manufactured by incorporating NaCl into DF water may have significantly lower levels of Ca present than retentate manufactured by incorporating NaCl into the final retentate; this will be explored in section 5.3.4.3.

Differences in pH upon reconstitution were also observed (Figure 20). It appeared that samples with lower solubility also had a lower pH upon reconstitution, and samples with higher solubility also had a higher pH upon reconstitution. A regression analysis of

pH (1h) vs. solubility (1h) revealed that pH was a statistically significant predictor of solubility ($p < 0.001$) (Appendix C).

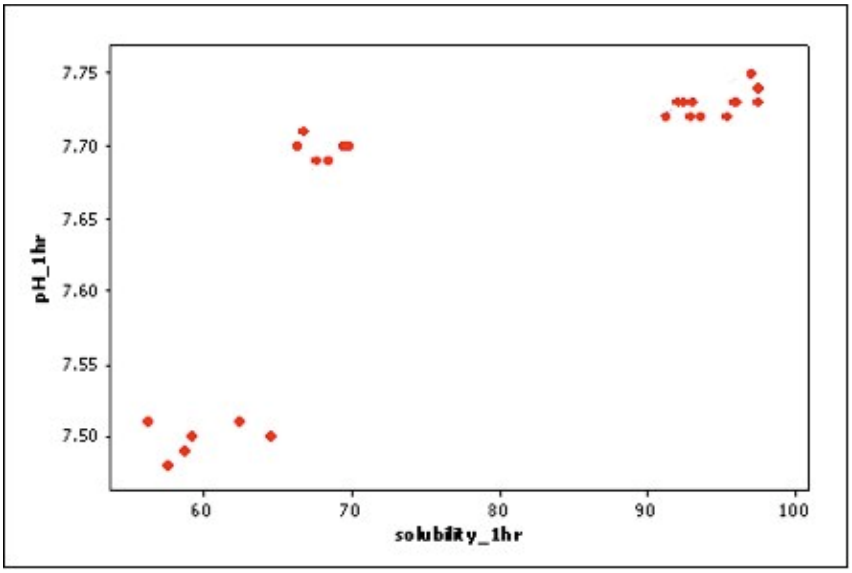

Figure 20: Scatterplot of solubility (1h) and pH (1h)

ANOVA, utilizing pH (1h) as response and treatment as fixed factor, indicated the existence of a statistically significant difference in pH due to the effect of NaCl treatment ($p < 0.001$) (Appendix C). With 99 % confidence, an increase from 0 mM NaCl to 50 mM NaCl in DF water was associated with a statistically significant ($p < 0.001$) mean pH increase by between 0.18 and 0.22. From 50 mM NaCl to 100 mM NaCl, a statistically significant ($p < 0.001$) mean pH increase by between 0.21 and 0.25 was observed. Taken together, these analyses indicated that additional experiments should be performed to separate the effect of pH on solubility from the effect of NaCl treatment on solubility. The results of these experiments are described in Section 5.2.5 and Section 5.2.6.

5.2.2. Three Hours Reconstitution

The data collected according the method outlined in section 4.3.1, pertaining to three hour reconstitution time, is shown in Appendix D. The statistical analysis of solubility on the laboratory stage mixer for one hour reconstitution time was conducted using the GLM command in Minitab according to Section 4.5.4. The interaction between block and treatment was not statistically significant at $\alpha = 0.01$, and thus the model was rerun without the interaction term. There was a statistically significant difference in solubility due to the effect of NaCl treatment ($p < 0.001$). Tukey's simultaneous comparisons are summarized below (Figure 21).

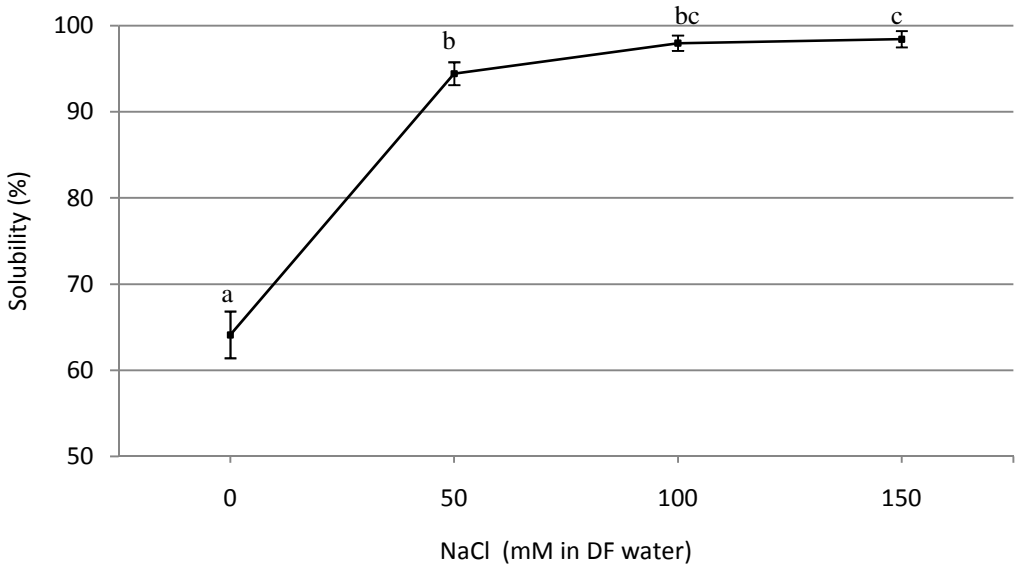

Figure 21: Mean solubility after three hours on laboratory stage mixer, treatment averaged across all blocks and replicates; different superscripts denote solubility results that are statistically significantly different from each other according to Tukey's simultaneous tests (Appendix D)

These results indicate that the addition of NaCl during DF steps significantly increased the solubility of the manufactured MPC when it was reconstituted for three hours. With 99 % confidence, the addition of 50 mM NaCl in DF water yielded a significant ($p < 0.001$) mean solubility increase by between 26.54 % and 34.13 %. The addition of 100 mM NaCl in DF water yielded a significant ($p < 0.001$) mean solubility increase by between 30.08 % and 37.66 %. The addition of 150 mM NaCl in DF water yielded a significant ($p < 0.001$) mean solubility increase by between 30.54 % and 38.12 %.

These results suggest that if a particular MPC is intended to be mixed for three hours at 22 °C, manufacturing the MPC using at least 50 mM NaCl in DF water may yield a significant improvement in solubility. The incorporation of 100 mM NaCl in DF water did not cause a significant improvement in solubility over that of 50 mM NaCl in DF water. However, if a food application demands the absolute highest possible MPC solubility (for example, a high-protein beverage containing MPC) , then a significant increase in solubility can be obtained by moving from a manufacture process incorporating 50 mM NaCl in DF water to one that incorporates 150 mM NaCl in DF water. No sensory studies were performed, so the sensory impact of the mean 4.0 % difference in MPC solubility (the difference between samples manufactured with 50 mM NaCl in DF water and 150 mM DF water, at 3h mixing time) cannot be discussed. However, future work may wish to explore the relationship between sensory perception and MPC solubility to determine the perceptibility of such differences in different food products.

A regression analysis was performed to further elucidate the connection between NaCl addition to DF water and the resulting increase in solubility. The tests associated with this analysis are located in Appendix D. The relationship between MPC solubility at 3 hours and NaCl concentration in DF water is described by the following equations: The equation for block 1 is: solubility_3hr = 65.0903 + 4.94575 log2(conc+1). The equation for block 2 is: solubility_3hr = 64.2566 + 4.94575 log2(conc+1). The equation for block 3 is: solubility_3hr = 64.2741 + 4.94575 log2(conc+1). The tests associated with this analysis are located in Appendix D, and the scatterplot of solubility at 3 hours vs. concentration is shown (Figure 22).

After adjusting for differences between the blocks, doubling the NaCl concentration is associated with an increase of between 4.48% and 5.41% in mean solubility with 99% confidence. This model would allow solubility to increase past 100% at NaCl concentrations above 132.3 mM. Therefore, any predictions above 132.3 mM are suspect and should be replaced with a value of 100%. Despite these problems, this model fits the data better than a quadratic fit. For comparison, the R^2 of the log fit was 97.9% versus 95.4% for the quadratic model. The quadratic model also begins to show a decrease in solubility at the highest concentration levels, which is not supported by the data.

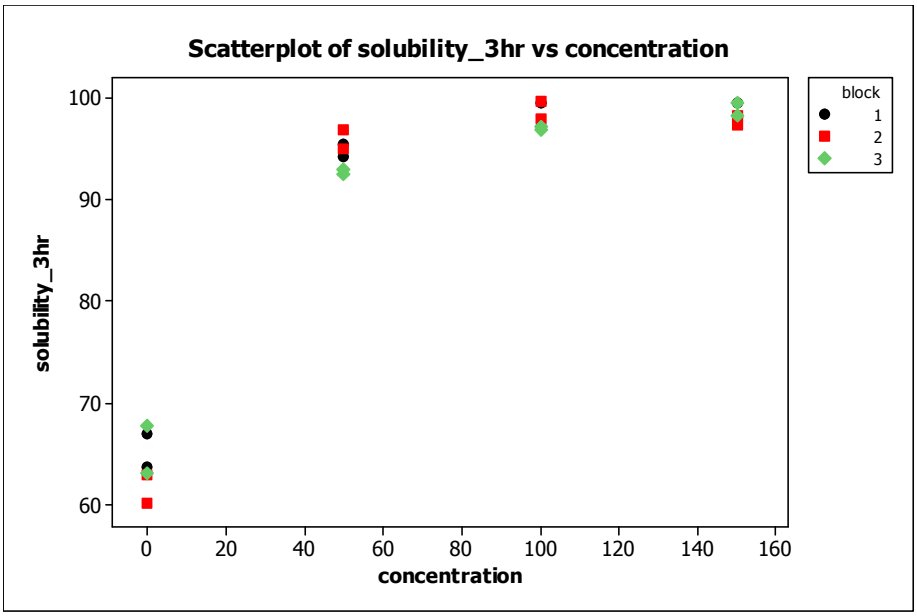

Figure 22: Scatterplot for solubility vs NaCl concentration (mM) at 3h

Similar to the solubility experiment performed at 1h reconstitution (section 5.2.1), differences in pH upon reconstitution were also observed (Figure 23) in this experiment. It appeared that samples with lower solubility also had a lower pH upon reconstitution, and samples with higher solubility also had a higher pH upon reconstitution. A regression analysis of pH (3h) vs. solubility (3h) revealed that pH was a statistically significant predictor of solubility ($p < 0.001$) (Appendix D).

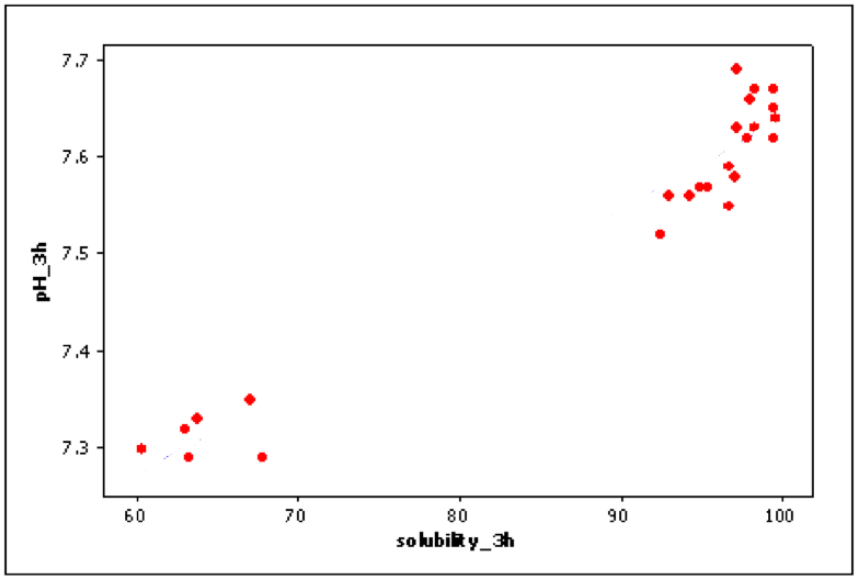

Figure 23: Scatterplot of solubility (3h) and pH (3h)

Furthermore, ANOVA, utilizing pH (3h) as response and treatment as fixed factor, indicated the existence of a statistically significant difference in pH due to the effect of NaCl treatment ($p < 0.001$). With 99 % confidence, the addition of 50 mM NaCl in DF water significantly increased ($p < 0.001$) pH by between 0.20 and 0.29. The addition of 50 mM NaCl to DF significantly increased ($p < 0.001$) pH by between 0.26 and 0.34. The addition of 150 mM NaCl in DF water significantly increased ($p < 0.001$) by between 0.30 and 0.39.

Taken together, these analyses, along with analyses conducted in Section 5.2.1, indicated that additional experiments should be performed to separate the effect of pH on solubility from the effect of NaCl treatment on solubility. The results of these experiments are described in Section 5.2.5 and Section 5.2.6. Moreover, because the sensory characteristics and texture properties of many food products which incorporate MPCs are highly dependent on pH (for example, process cheese and high-protein

beverages) the differences in pH must be accounted for and controlled when utilizing MPCs manufactured with NaCl in DF water.

5.2.3. Insolubility Index

The data collected according the method outlined in Section 4.3.2 is shown in Appendix E. In this experiment, as well as all subsequent solubility experiments, n = 12 (no internal replication was conducted). The statistical analysis of solubility according to ISI was conducted using GLM command in Minitab according to Section 4.5.4. At α = 0.01, there was a statistically significant difference in solubility due to the effect of NaCl treatment ($p < 0.001$). Tukey's simultaneous comparisons are summarized below (Figure 24).

This test examines the performance of MPC under conditions of low mixing time (30 sec) but high shear (in the form of blending at 3800 rpm for 90 sec.), followed by a period of potential particle settling. This differs from the methods used previously, which examined MPC under conditions of mixing at 960 rpm for 1h and 3h, because high shear rates may assist in breaking large particles, possibly facilitating the rehydration process. These results indicate that the addition of NaCl during DF steps significantly decreased the amount of sediment remaining after the ISI method was completed. With 99 % confidence, the addition of 50 mM NaCl in DF water significantly decreased ($p < 0.004$) mean sediment by between 5.53 ml and 0.50 ml. The addition of 100 mM NaCl in DF water significantly decreased ($p < 0.001$) mean sediment by between 10.00 ml and 4.97 ml The addition of 150 mM NaCl in DF water significantly decreased ($p < 0.001$) mean sediment by between 12.00 ml and 6.97 ml, but the difference in mean sediment between

the 100 mM and 150 mM treatment levels was not found to be statistically significant ($p = 0.029$).

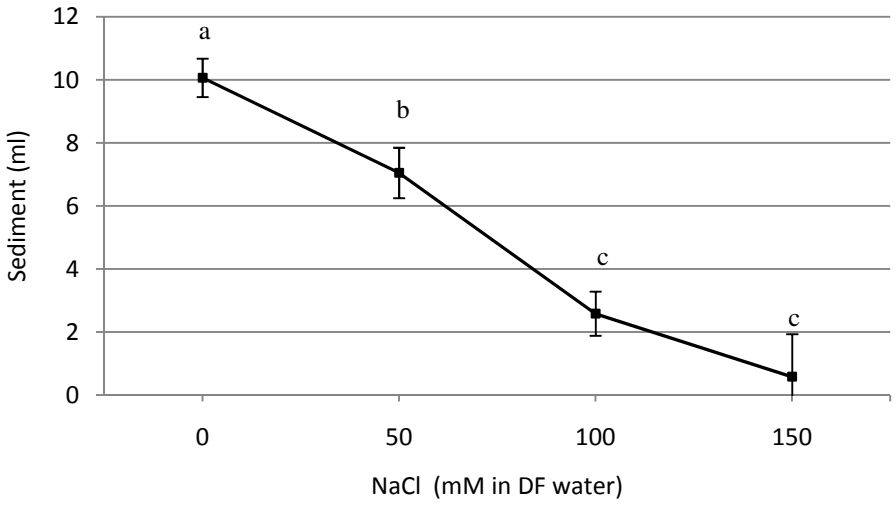

Figure 24: Mean sediment (ml) recorded after performing ISI method, treatment averaged across all blocks and replicates; different superscripts denote ISI results that are statistically significantly different from each other according to Tukey's simultaneous tests (Appendix E)

These results suggest that the manufacture of MPC incorporating up to 100 mM NaCl in DF water may significantly reduce the amount of sediment present, after mixing under conditions of high shear and low time. To further reduce the amount of sediment remaining in a 5 % w/w solution of MPC, it may be possible to move from an MPC manufacture process incorporating 100 mM NaCl in DF water to one which incorporates 150 mM NaCl in DF water, but the reduction may not be significant enough to warrant the increased consumption of NaCl and associated costs.

A regression analysis was performed to elucidate the connection between NaCl addition to DF water and the resulting decreases in ml sediment. With 99% confidence, for every 1 mM increase in NaCl added to DF water, there was a decrease in the amount

of sediment between 0.076 ml and 0.056 ml. The tests associated with this analysis are located in Appendix E. The relationship between MPC solubility as determined by ISI and NaCl concentration in DF water is described by the following equations: The equation for block 1 is: ISI = 9.4875 - 0.0658333(concentration). The equation for block 2: ISI = 10.1875 - 0.0658333(concentration). The equation for block 3 is: ISI = 10.35 - 0.0658333(concentration). It must be noted that predictions at or near 150 mM may be below 0 ml sediment. Therefore, this regression model should be used for descriptive purposes, and not for predictions at the highest levels.

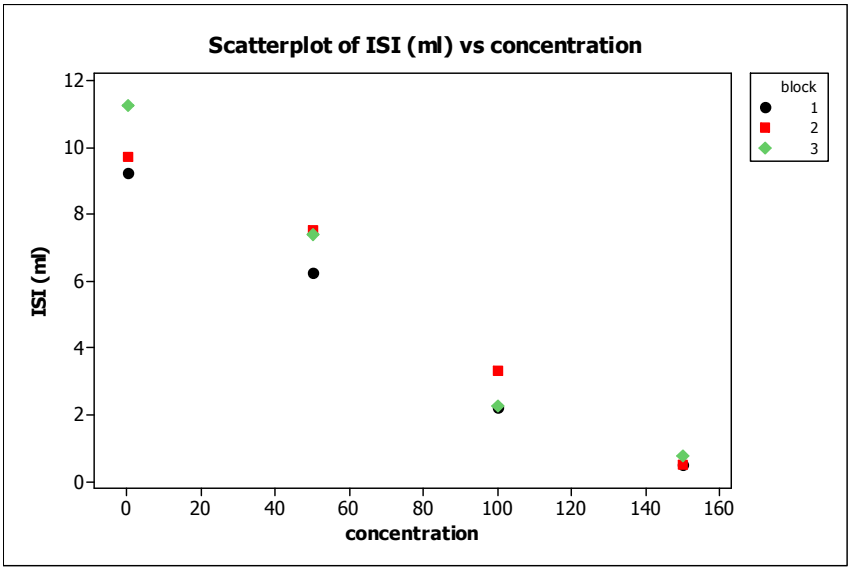

Figure 25: Scatterplot for solubility according to ISI and NaCl concentration

5.2.4. Solubility using method of Anema et al. (2006)

The data collected according the method outlined in Section 4.3.3 is shown in Appendix F. The statistical analysis of solubility according to the method of Anema et al

(2006). was conducted using GLM command in Minitab according to Section 4.5.4. At $\alpha = 0.01$, there was a statistically significant difference in solubility due to the effect of NaCl treatment ($p < 0.001$). Mean solubility and significant differences from Tukey's simultaneous comparisons are summarized below (Figure 26)

These results indicate that the addition of NaCl during DF steps significantly increased the solubility of the manufactured MPC under these mixing conditions when at least 100 mM NaCl was added. With 99 % confidence, the addition of 50 mM NaCl to DF water did not yield a significant increase in mean solubility ($p = 0.060$). The addition of 100 mM NaCl to DF water significantly increased ($p < 0.002$) mean solubility of MPC by between 20.09 % and 59.08 %. The addition of 150 mM NaCl to DF water yielded a significant increase ($p = 0.001$) in mean solubility of MPC, but no significant differences ($p = 0.128$) were found between the 100 mM and 150 mM treatment levels.

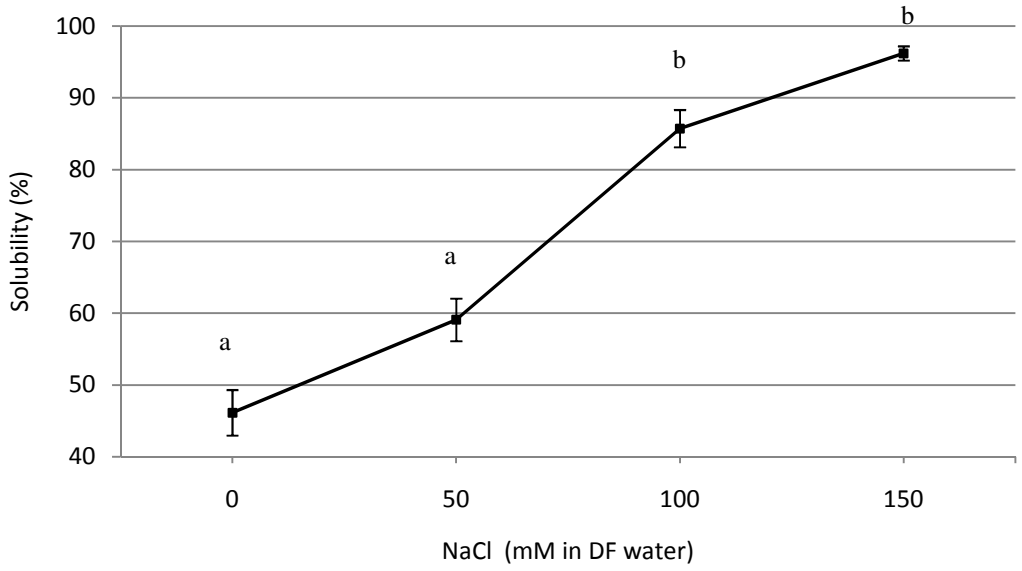

Figure 26: Mean solubility (%) recorded after performing solubility method of Anema et al. (2006), treatment averaged across all blocks and replicates; different superscripts denote solubility results that are statistically significantly different from each other according to Tukey's simultaneous tests (Appendix F).

This test examines the performance of MPC under conditions of 30 min mixing time using an overhead stirrer, the most likely equipment to be used in a food manufacturing facility setting. The results suggest that if a 5% w/w MPC solution is mixed for 30 min. using an overhead stirrer at a speed of 600 rpm using a propeller blade 4.4 cm in diameter, then in order to obtain a significant increase in MPC solubility, that MPC should be manufactured with at least 100 mM NaCl incorporated into DF water. MPC manufactured with 50 mM incorporated into DF water did not perform significantly better than MPC manufactured with no NaCl incorporated into DF water, and MPC manufactured with 150 mM NaCl incorporated into DF water did not perform

significantly better than MPC manufactured with 100 mM NaCl incorporated into DF water.

A regression analysis was performed to further elucidate the connection between NaCl addition to DF water and the resulting increase in solubility. With 99% confidence, the addition of 1 mM NaCl to DF water resulted in an increase in solubility between 0.28% and 0.42%. The tests associated with this analysis are located in Appendix F. The relationship between MPC solubility according to the method of Anema et al. (2006) and NaCl concentration in DF water is described by the following equations. The equation for block 1 is: solubility = 45.66 + 0.353667(concentration). The equation for block 2 is: solubility = 45.215 + 0.353667(concentration). The equation for block 3 is: solubility = 44.905 + 0.353667(concentration).

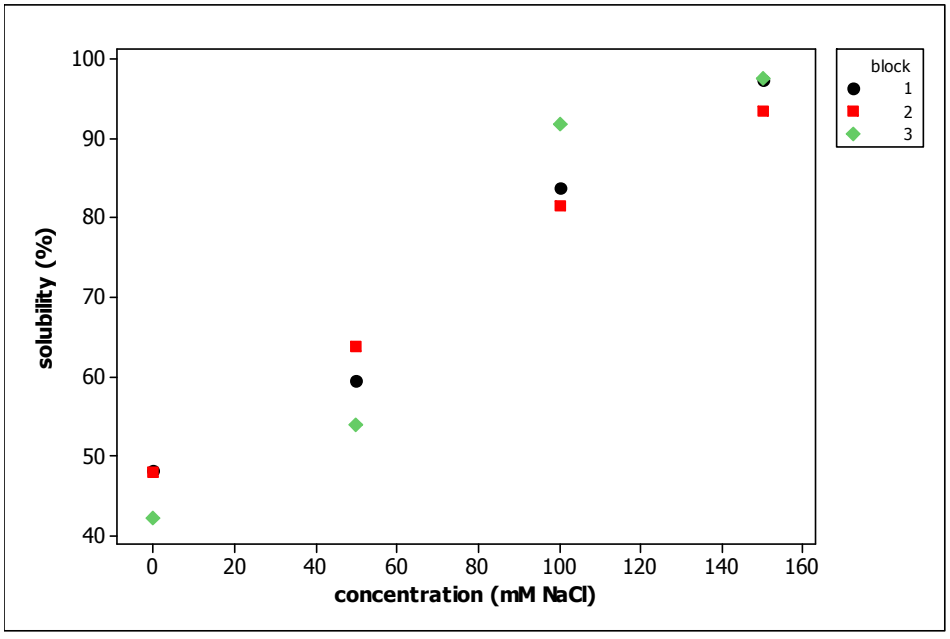

Figure 27: Scatterplot for solubility according to the method of Anema et al. (2006).

5.2.5. After Adjustment to Treatment Level 4 (150 mM NaCl in DF water) pH

A correlation between pH and solubility, and pH and NaCl treatment, was suggested by experiments discussed in sections 5.2.1 and 5.2.2. Therefore, the following experiments were conducted to determine the effect of pH adjustment on the solubility of MPC. At treatment 4 pH (7.68 ± 0.02), with 99 % confidence there is a significant increase ($p < 0.001$) in mean solubility, by between 23.16 % and 40.70 %, when MPC was manufactured with 150 mM NaCl in DF water. However, there were no significant differences in solubility between MPCs manufactured with 50 mM, 100 mM, or 150 mM NaCl incorporated into DF water.

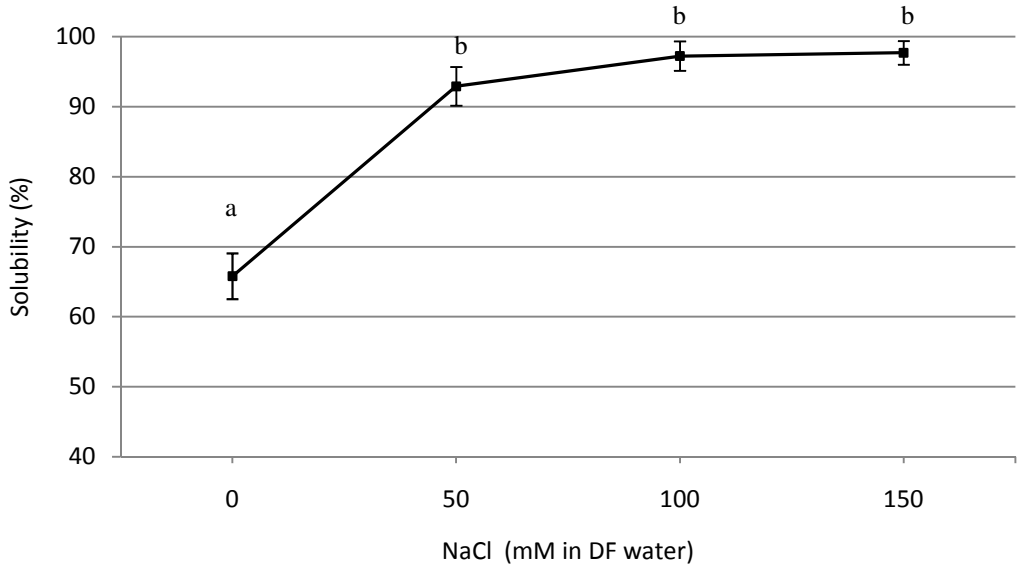

Figure 28: Mean solubility after adjustment to treatment level 4 pH, after reconstitution on laboratory stage mixer (3h); treatment averaged across all blocks; different superscripts denote solubility results that are statistically significantly different from each other according to Tukey's simultaneous tests (Appendix G)

5.2.6. After Adjustment to Treatment Level 1 (0 mM NaCl in DF water) pH

At treatment 1 pH (7.41 ± 0.02), there is a significant increase in solubility in MPC samples which were manufactured with NaCl incorporated into DF water. With 99 % confidence, the addition of 50 mM NaCl in DF water significantly increased ($p < 0.001$) the mean solubility of MPC by between 12.75 % and 28.28 %. The addition of 100 mM NaCl in DF water significantly increased ($p < 0.001$) the mean solubility of MPC by between 25.87 % and 41.39 %. The addition of 150 mM NaCl in DF water significantly increased ($p < 0.001$) the mean solubility of MPC by between 27.83 % and 43.36 % , but the difference between the 100 mM and 150 mM treatment levels was not significant ($p = 0.617$).

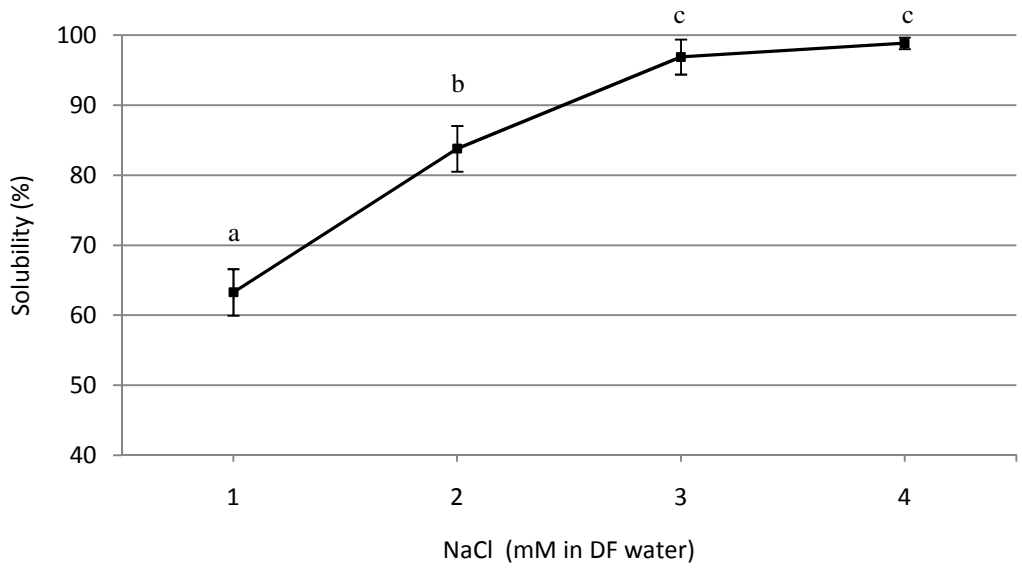

Figure 29: Solubility after adjustment to Control pH, after reconstitution on laboratory stage mixer (3h); treatment averaged across all blocks and replicates; different superscripts denote solubility results that are statistically significantly different from each other according to Tukey's simultaneous tests (Appendix H)

5.3. Proximate Analysis of Material Obtained During MPC Manufacture

5.3.1. FTIR Analysis

Proximate analysis (fat, protein, casein, lactose, TS) of skim milk and all products obtained by UF and DF are displayed in Appendix I: Proximate Analysis as Determined by FOSS Milkoscan FT2. A comparison between the Milkoscan FT2 data and protein data obtained by Elementar rapid N is conducted in section 5.3.2

There are no previous studies reported in the literature involving the proximate analysis of skim milk and all products obtained by UF and DF by Milkoscan FT2. Yet, the instrument's speed, lack of lengthy sample preparation requirements, simplicity of user operation, and ability to analyze several compositional constituents simultaneously have great potential use in the manufacture of retentate. The data is largely consistent with what is expected to occur during the MPC manufacture process; protein, casein, and fat are concentrated and thus consist of higher proportions of the total solids as the manufacturing process continues. Conversely, the majority of lactose is removed during the DF1, DF2, and DF3 manufacturing steps, which is consistent with previous observations (Mistry and Hassan, 1991a).

The data shown provides evidence that Milkoscan FT2 equipment serves as a valuable aid during the MPC manufacture process. Its ability to provide near real-time analysis of a wide variety of constituents would be valuable to MPC manufacturers and would likely lead to the manufacture of retentate with more consistent protein, lactose, fat, and total solids. This, in turn, could lead to the manufacture of more consistent spray-dried MPCs.

5.3.2. Elementar rapid N nitrogen analysis

5.3.2.1. Protein Content of Skim Milk, UF, and DF Products

Protein content of all skim milks, along with all products obtained from UF and DF, as obtained by elementar rapid N, are shown in Appendix J. Comparison of protein Milkoscan FT2 data with Elementar rapid N data reveal that the two methods are statistically significantly different from each other. This comparison is shown below (Figure 30). Output from statistical analysis is shown in Appendix K, and the data is further discussed in section 5.3.3.

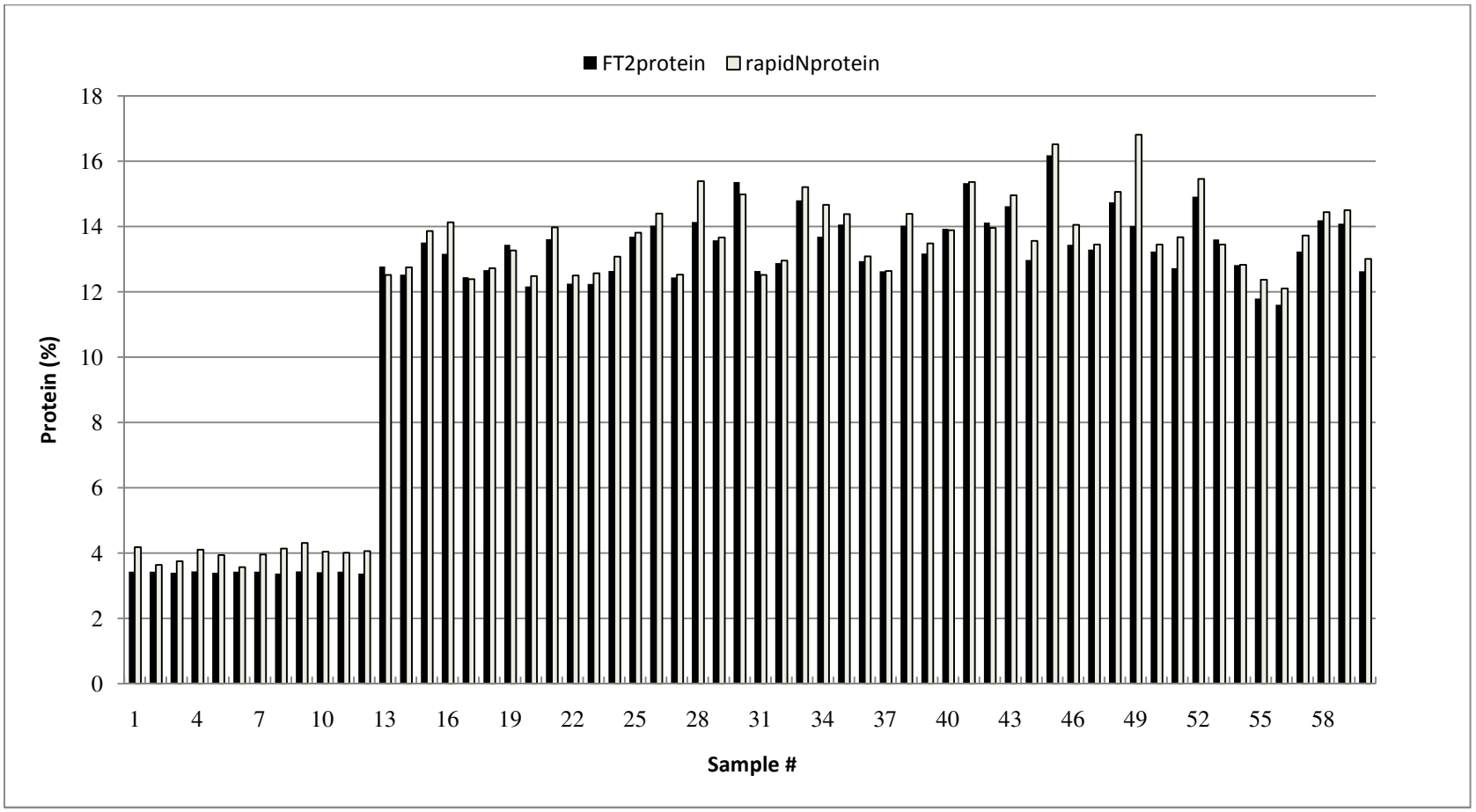

Figure 30: Comparison of protein values obtained by Milkoscan FT2 and those obtained by rapid N. X axis represents sample measured, Milk Block 1 Treatment 1 representing Sample 1, and DF3 Block 3 Treatment 4 representing Sample 60.

5.3.2.2. Protein Content of Dried MPC

Protein content of all dry MPCs, as determined by Elementar rapid N and collected according to the method outlined in Section 4.4.2, is shown in Appendix J. The statistical analysis of the protein content of all dry MPCs was conducted using the GLM command in Minitab according to Section 4.5.4. At $\alpha = 0.01$, there was a statistically significant difference in protein content due to the effect of NaCl treatment ($p = 0.003$). Mean protein % and significant differences from Tukey's simultaneous comparisons are summarized below (Figure 31).

When more than 100 mM NaCl was added to DF water, there was a statistically significant decrease in protein content of dry MPC. From a mean protein content of 84.99 % (control), the addition of 100 mM NaCl to DF water was associated with a decrease in protein content between 0.33 % and 6.47 %. From the control, the addition of 150 mM NaCl to DF water was associated with a decrease in protein content between 0.38 % and 6.52 %.

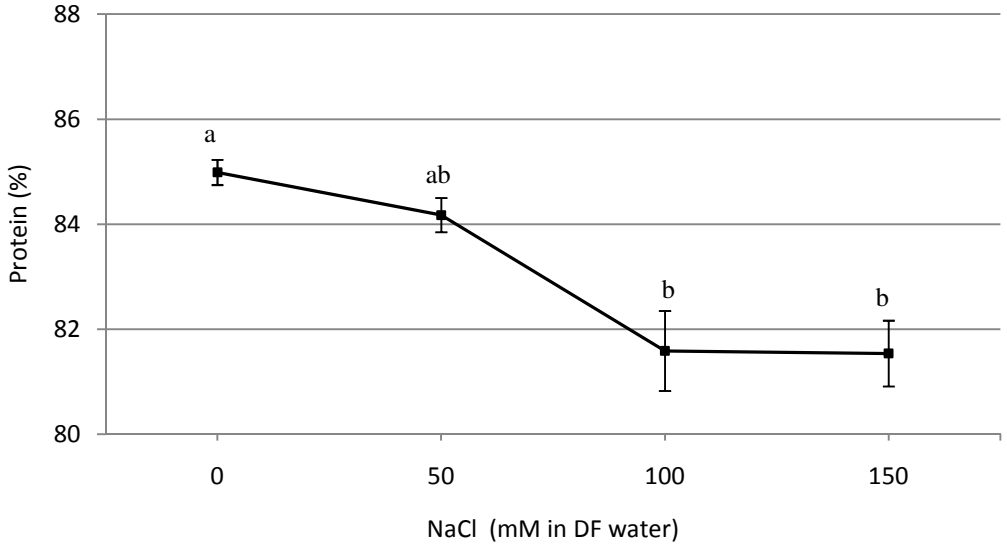

Figure 31: Protein analysis of dry MPC by Elementar rapid N, treatment averaged across all blocks; different superscripts denote protein results that are statistically significantly different from each other according to Tukey's simultaneous tests (Appendix J).

5.3.3. Comparison of Milkoscan FT2 and Elementar rapid N protein analysis

As no prior MPC studies have reported protein measurements of all UF and DF products obtained by FOSS Milkoscan FT2 equipment, protein measurements obtained by this method were compared with those obtained by Elementar rapid N. Protein data obtained by FOSS Milkoscan FT2 and Elementar rapid N were compared according to the statistical procedure outlined in section 4.5.4. The SAS output is displayed in Appendix K. Protein data obtained by both methods is displayed visually in Figure 30.

At $\alpha = 0.01$, there is a statistically significant difference ($p < 0.001$) between protein content as measured by the two analysis methods, though no additional analyses were performed to determine which of the two methods yielded the overall higher protein measurements.

There could be several reasons why the two measurement methods differed in a statistically significant fashion. The first reason stems is that the two analysis methods measure fundamentally different sample characteristics. The Elementar rapid N combusts the sample, traps the resulting vapor, and filters it through a drying chamber and reduction chamber before this passing vapor (which should only contain nitrogen at this point) to a detector. The resulting voltage measurements are then processed and converted to a raw nitrogen measurement, and then, according to the Dumas method, to a protein measurement. A slightly high protein measurement may result if either the sample contains a high amount of non-protein nitrogen, or the drying chamber or reduction chamber fail to trap virtually all vapor that is not nitrogen. Though the instrument was calibrated according to manufacture's specification for the analysis of dairy products (section 4.4.2), it is not unfeasible that, like most precision laboratory equipment, instrument accuracy may degrade over time. The FOSS Milkoscan FT2, on the other hand, measures protein content by obtaining an FTIR spectra of the sample and probing this spectra in the amide I and amide II regions (1700 to1400 cm^{-1}) and the fingerprint region (1800 to 700 cm^{-1}). The Milkoscan FT2 instrument is calibrated for the analysis of dairy products by the use of standards as described in section 4.4.1. Despite the existence of standards for concentrated milk, there do not exist standards for milk concentrated by UF and DF processes (possibly because physical and chemical properties, such as fluid viscosity, color, and mineral content of the UF and DF processed samples can change depending upon the pH and temperature at which these concentration processes are carried out).

Despite the statistically significant differences observed in protein measurements obtained between the two processes, for the purposes of this experiment the protein data obtained by both seem to be in relative agreement. This study utilized FOSS Milkoscan FT2 data in an attempt to verify expected increases in protein concentration (on dry basis) that should occur as the UF and DF concentration processes took place, as the ability to obtain this information within minutes (and potentially in line if the correct equipment is engineered) could be a huge benefit to an MPC manufacturer However, it is clear that, from. rapid N protein data obtained, that FTIR application to the measurement of protein content in UF and DF retentates in a production environment, where product consistency is of utmost importance, may require additional study, as the both methods were not strictly in agreement with each other.

5.3.4. Minerals analysis

5.3.4.1. Mineral Content of Skim Milk, UF, and DF Products on Wet Basis

Data obtained from minerals analysis (Ca, Mg, K, and Na) of skim milk and all products obtained by UF and DF, as determined by ICP-MS, as well as SAS output, are displayed in Appendix L, while the means for Ca, Mg, K, and Na are displayed below. Statistical analysis of the difference in Ca, Mg, K, and Na content at DF1, DF2, and DF3 process levels from UF process levels was analyzed using distinct PROC MIXED statements as described in Section 4.5.4. At $\alpha = 0.01$, there is no statistically significant difference in Ca level due to process ($p = 0.199$) or treatment ($p = 0.188$).

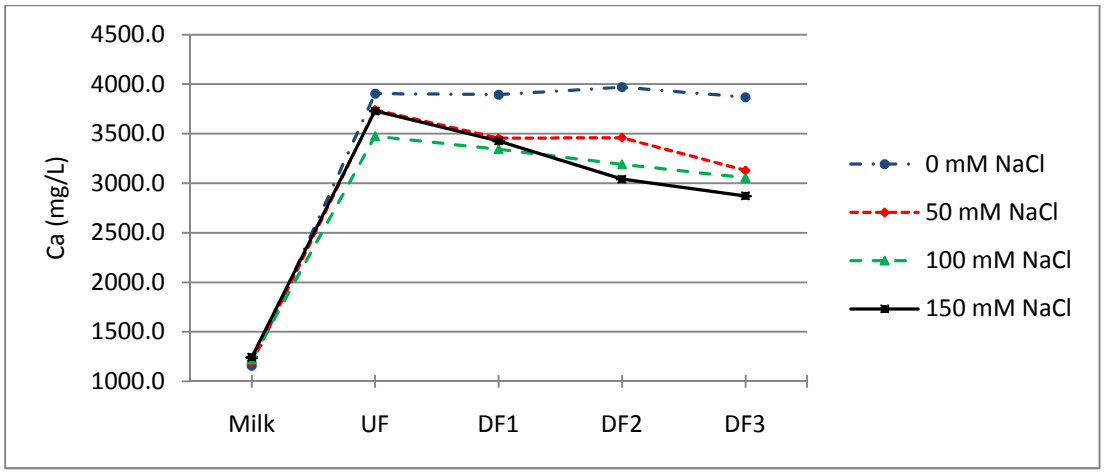

Figure 32: Mean Ca (mg/L) of skim milk and all products obtained by UF/DF processes on wet basis

Although no significant differences in Ca content were found in this particular study, this data is generally consistent with what has been previously reported concerning the effect of mineral addition on the Ca system in milk (Dalgleish and Parker, 1980, Dickson and Perkins, 1971, Horne, 1998). According to the previously established work, as well as work which attempts to model ionic calcium as a function of ionic strength (Mekmene et al., 2009), as ionic strength of the milk solution increases, a larger percentage of total calcium will be found in the ionic phase. Ionic-phase minerals are then able to be removed by the UF/DF processes, which should lead to an overall lower level of Ca in the final UF/DF product. This general trend is illustrted by the plot of Ca (mg/ml) by treatment level and process (Figure 32). However, possible causes of failing to find a significant difference in Ca levels may involve temperature variations that occurred during UF and DF processes, as well as differences in the concentrations of products at similar processing levels. Future work should take care to control these two variables, as they may highly impact levels of Ca present.

At $\alpha = 0.01$, there is a statistically significant difference in Mg level due to process ($p = 0.001$) and Treatment ($p = 0.004$). In terms of difference in Mg content, Tukey's comparisons state that, on average, the increase in Mg from UF to DF3 was statistically larger than the increase in Mg from UF to DF1. Also, on average, treatment level 1 had a significantly larger increase in Mg between UF and DF than treatment level 4.

Milk contains roughly 130 mg/L Mg, and of that amount roughly 33 % is found in the colloidal phase (Fox, 1989); yet, this association may shift as minerals are added to the milk system and overall ionic strength of the milk system increases (Walstra et al., 2006). The observation that treatment level 1 had a significantly larger increase in Mg between UF and DF than treatment level 4 is generally consistent with previous observations relating an increase in ionic strength to increased solubility of milk salts (Mekmene et al., 2009).

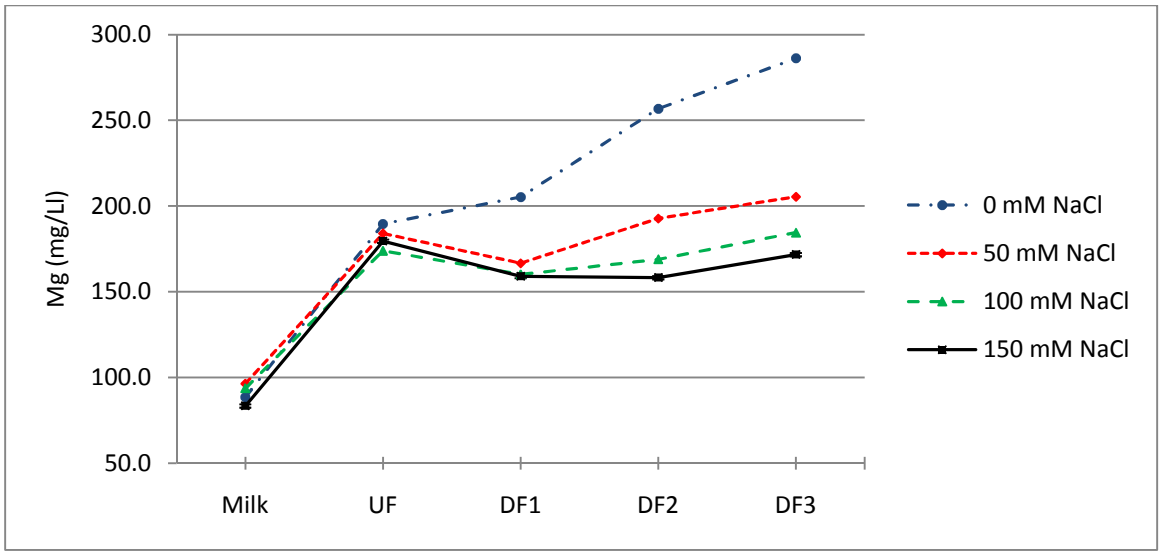

Figure 33: Mean Mg (mg/L) of skim milk and all products obtained by UF/DF processes on wet basis

At $\alpha = 0.01$, there is a statistically significant difference in the difference in K content due to process ($p < 0.001$). As nearly roughly 92 % of K in milk exists in the ionized form (Fox, 1989), it would be expected to be removed during DF. The sharp drops in K during DF1 are consistent with this data, however, the K results presented here stand in contrast to data collected by Floris et al., (2007) who noted variation in K levels in MPCs collected domestically and internationally. Taken together, these observations suggest that, unless manipulated during the manufacture process, K levels will likely drop, and that K levels are not affected by the addition of NaCl into DF water during the MPC manufacture process. There is no statistically significant difference in K content due to treatment ($p = 0.222$), indicating that the level of NaCl treatment did not have a significant effect on the concentration of K present.

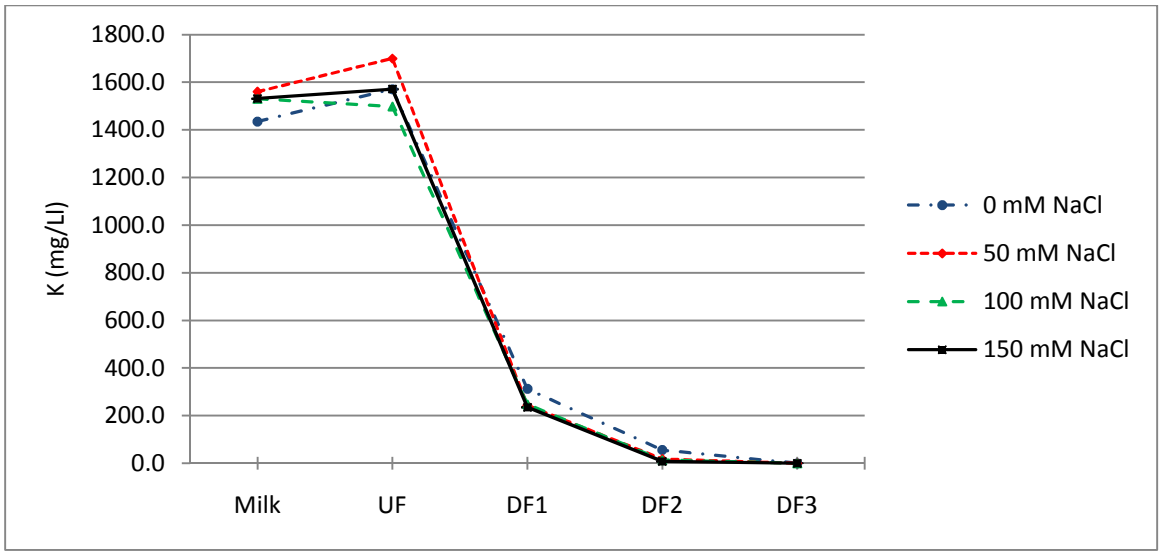

Figure 34: Mean K (mg/L) of skim milk and all products obtained by UF/DF processes on wet basis

At $\alpha = 0.01$, there is a statistically significant difference in Na content due to treatment ($p < 0.001$). The increase in Na content from UF to DF, on average, was significantly higher in treatments 2, 3, and 4 (50, mM, 100mM and 150 mM NaCl incorporated into DF water, respectively) than in treatment 1 (control), as well as significantly higher in treatment 4 than in treatment 2. This indicates that, as between 50 mM NaCl and 150 mM NaCl were incorporated into DF water, progressively higher levels of NaCl were found in retentate. There was no statistically significant difference in Na content due to process ($p = 0.052$).

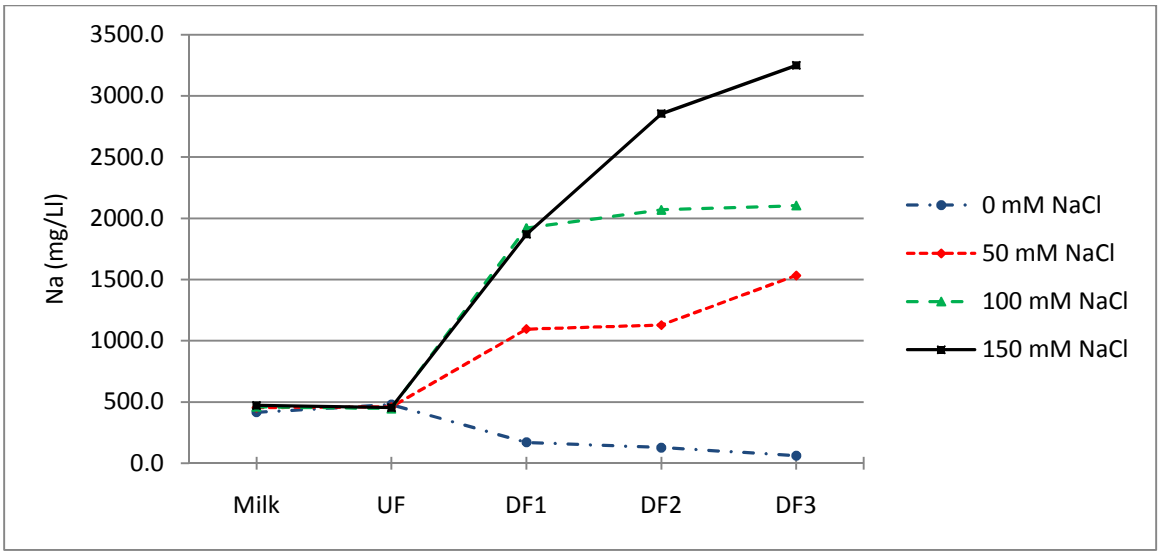

Figure 35: Mean Na (mg/L) of skim milk and all products obtained by UF/DF processes on wet basis

5.3.4.2. Mineral Content of Skim Milk, UF, and DF Products on Dry Basis

Data obtained from minerals analysis (Ca, Mg, K, and Na) of skim milk and all products obtained by UF and DF, as determined by ICP-MS, as well as SAS output, are displayed in Appendix L, while the means for Ca, Mg, K, and Na are displayed below. Statistical analysis of the difference in Ca, Mg, K, and Na content at DF1, DF2, and DF3 process levels from UF process levels was analyzed using distinct PROC MIXED statements as described in Section 4.5.4.

At $\alpha = 0.01$, there was no statistically significant ($p = 0.013$) difference in Ca content. This result is consistent with Ca results for wet basis (section 5.3.4.1) all previous discussion concerning Ca results for wet basis also applies here. It should be noted that dry basis Ca analysis may more accurately reflect the differences in the true proportion of Ca to solid material, especially if the MPC manufacture process had not been balanced according to the weight of water removed. Mean Ca content on dry basis is

illustrated in Figure 36. No statistically significant interaction was found between process and treatment ($p = 0.343$).

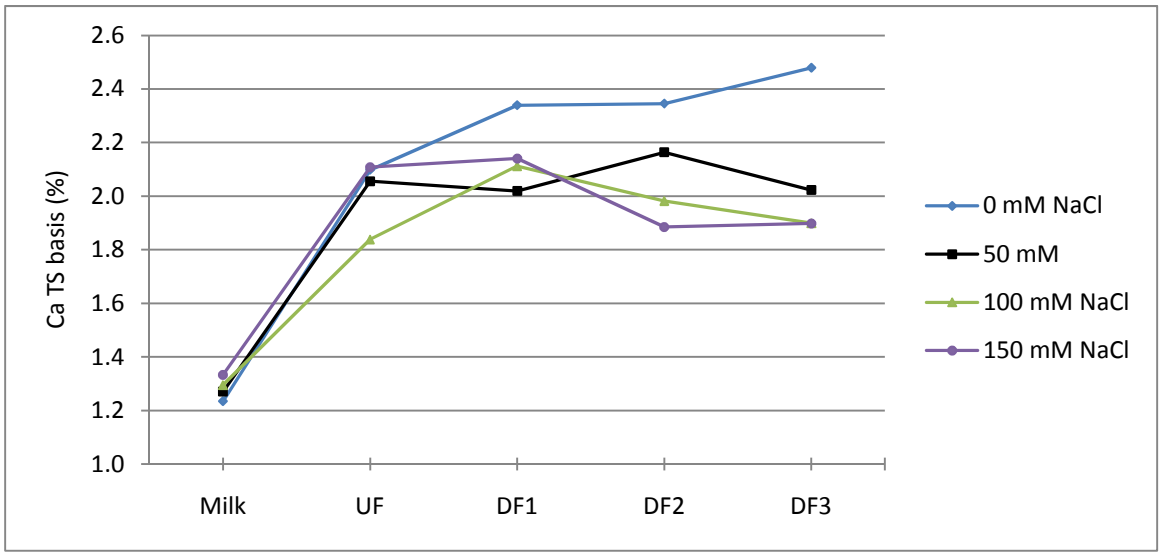

Figure 36: Mean Ca (%) of skim milk and all products obtained by UF/DF processes on dry basis

At $\alpha = 0.01$, there is a statistically significant difference in Mg level due to process ($p = 0.001$) but not due to treatment ($p = 0.239$). There was no statistically significant interaction between process and treatment ($p = 0.023$). Mean Mg content on dry basis is illustrated in Figure 37. This differs somewhat from the wet basis analysis, in which Mg levels were statistically significantly different due to both process and treatment. The differences observed in the wet basis analysis could have been due to differences in the final concentration factor achieved, though this was not explicitly tested.

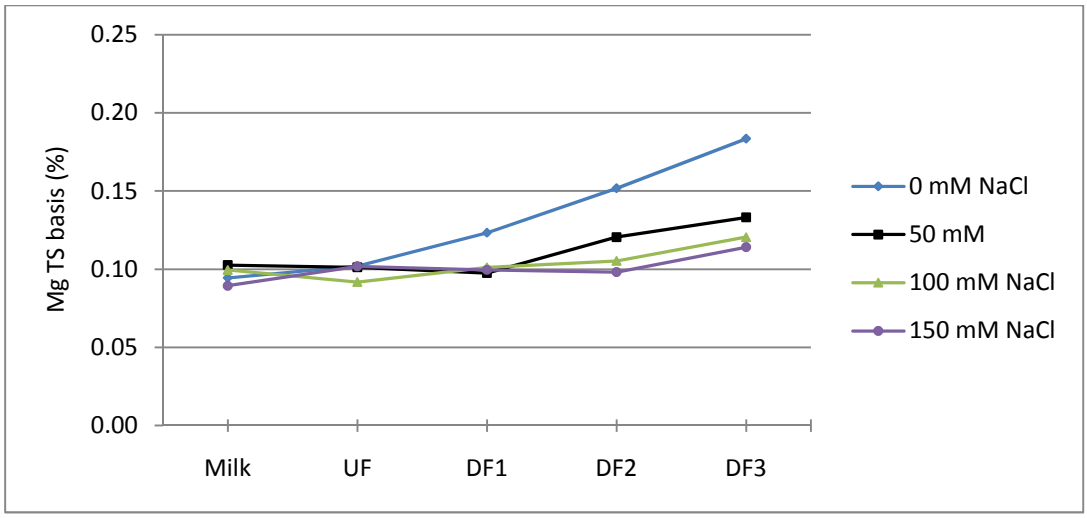

Figure 37: Mean Mg (%) of skim milk and all products obtained by UF/DF processes on dry basis

At $\alpha = 0.01$, there is a statistically significant ($p < 0.001$) difference in K content due to process, but not due to treatment ($p = 0.229$) or process by treatment interaction ($p = 0.174$). Mean K content is illustrated by Figure 38. This result is consistent with the result for wet basis K content (section 5.3.4.1).

At $\alpha = 0.01$, there is a statistically significant ($p < 0.001$) difference in Na content due to treatment, but not process ($p < 0.014$) or process by treatment interaction ($p = 0.079$). Mean Na content is illustrated by Figure 39. This result is consistent with the result for wet basis Na content (section 5.3.4.1).

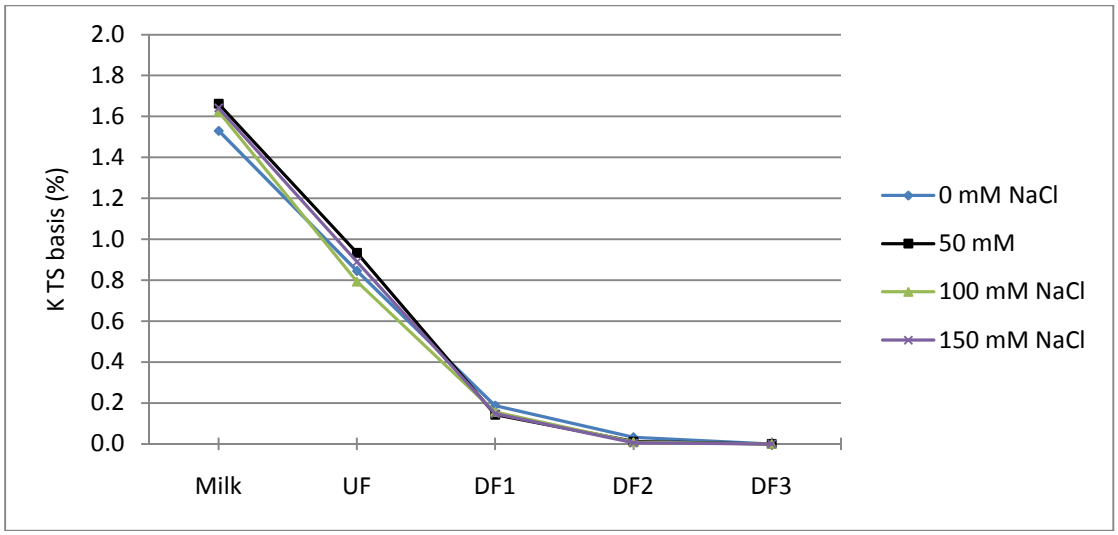

Figure 38: Mean K (%) of skim milk and all products obtained by UF/DF processes on dry basis

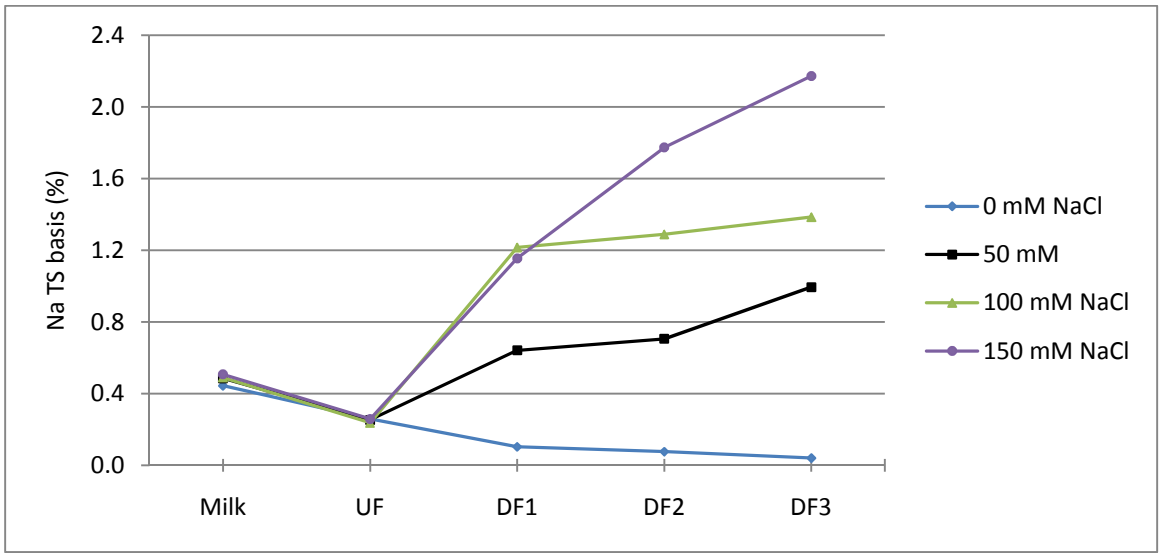

Figure 39: Mean Na (%) of skim milk and all products obtained by UF/DF processes on dry basis

It is also noted that on dry basis in the DF3 product, there are significant increases in the ratio of Ca to Mg as treatment level increases. This relationship is described by a regression of Ca/Mg against the mean-centered predictor NaCl concentration. The relationship between the ratio of Ca to Mg in DF3 is described by the following

equations. The equation for block 1 is: CaMgRatio = 16.3193 + 0.021788(conc-75) - 0.000143511(conc-75^2). The equation for block 2 is: CaMgRatio = 15.1105 + 0.021788(conc-75) - 0.000143511(conc-75^2). The equation for block 3 is: CaMgRatio = 16.3922 + 0.021788(conc-75) - 0.000143511(conc-75^2). Minitab output is located in Appendix M, and a scatterplot of the ratio of Ca to Mg in DF3 on dry basis vs. NaCl concentration is shown (Figure 40).

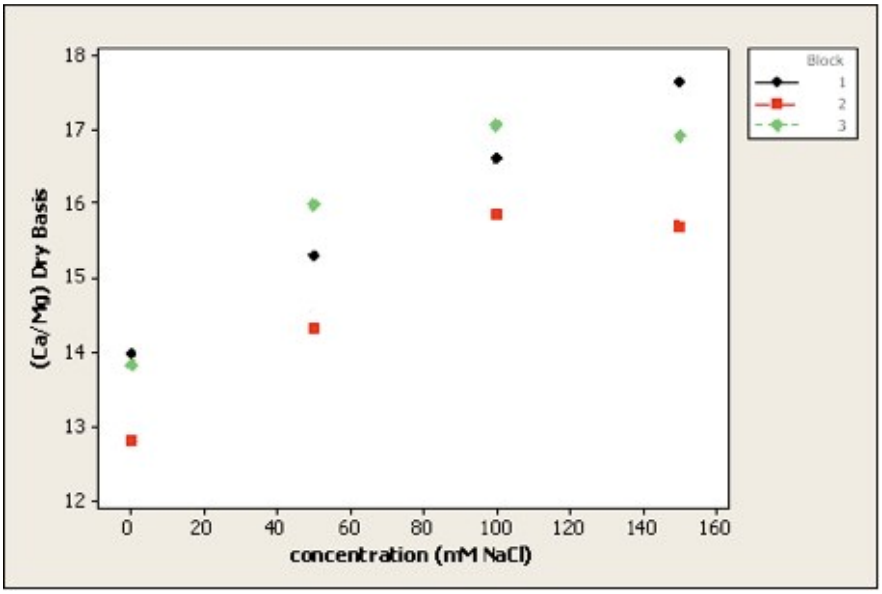

Figure 40: Scatterplot of the ratio of Ca to Mg in DF3 vs. NaCl concentration

5.3.4.3. Mineral Content of Dry MPC

Mineral content of all dry MPCs, as determined by ICP-MS and collected according to the method outlined in section 4.4.3, is shown in Appendix N. The statistical analysis of the mineral content of all dry MPCs was conducted using the General MANOVA command in Minitab according to section 4.5.4. Factors tested were Block and Treatment. Responses were Ca, Mg, and Na; because K was below detection limits in

all dry MPC, K values were considered to be zero and excluded from the analysis. At $\alpha = 0.01$, there was a statistically significant difference in mineral content due to the effect of Treatment according to both Wilks' and Lawley-Hotelling ($p < 0.001$) criterion. Examination of univariate ANOVA for each mineral revealed statistically significant differences in Na content ($p < 0.001$) and Mg content ($p < 0.001$) due to treatment. Ca content was not statistically significantly different due to Treatment ($p = 0.016$). Averages across all blocks are shown (Figure 41).

ANOVA indicated that there were significant differences ($p < 0.001$) in powder Na content at all treatment levels. With 99 % confidence, the addition of 50 mM NaCl in DF water significantly increased ($p < 0.001$) the mean Na content of MPC by between 2.48 mg/g and 7.44 mg/g. The addition of 100 mM NaCl in DF water significantly increased ($p < 0.001$) the mean Na content of MPC by between 5.80 mg/g and 10.75 mg/g. The addition of 150 mM NaCl in DF water significantly increased ($p < 0.001$) the mean Na content of MPC by between 9.57 mg/g and 14.53 mg/g.

It is also observed that one of the MPCs manufactured during this experiment contain similar levels of Na as a typical sodium caseinate. Due to variations in alkali requirement during sodium caseinate manufacture, sodium caseinate sodium content may typically vary between 0.99 % to 1.75 % (Mulvihill, 1989). MPC manufactured at treatment level 2 contains a mean Na value of 0.50 %, treatment level 3 contains a mean Na value of 0.83 %, and treatment level 4 contains a mean Na value of 1.21 %, respectively, on average. This indicates that the manner in which MPCs were manufactured during this experiment may bear similarities with the exchange of Na for Ca which takes place in sodium caseinate manufacture as the acidified, calcium-depleted

curd is then washed and solubilized in a weak NaOH solution. There is potential for future work to verify the similarity of these two mechanisms.

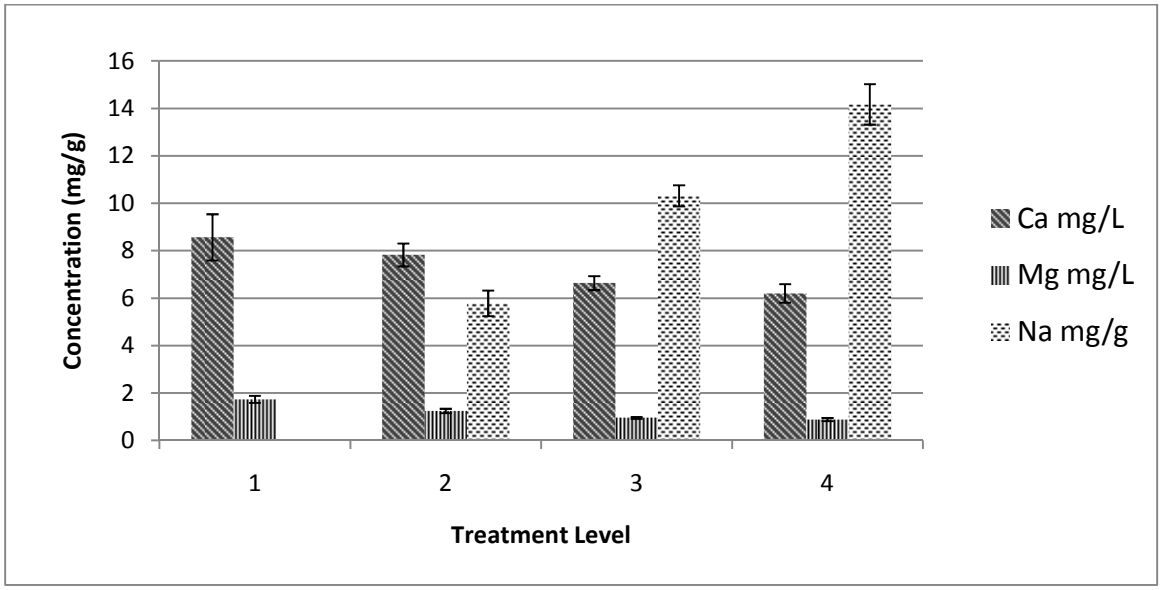

Figure 41: Ca, Mg, and Na analysis of dry MPC by ICP-MS, Treatment averaged across all blocks

The amount of Na present in the final MPCs, and the solubility results reported in this study, are in agreement with values reported in patent literature. Carr et al. (2002) described a monovalent cation addition procedure in which the final powder, which exhibited enhanced solubility over powders which had not been replenished with a monovalent cation, contained between 0.013 and 0.300 mol Na per 100 g protein. The MPC manufactured at treatment levels 2, 3, and 4 contain concentrations of Na per 100 g protein falling between these values. The MPC manufactured at treatment level 2 contains on average 0.026 mol Na per 100 g protein, the MPC manufactured at treatment level 3 contains on average 0.044 mol Na per 100 g protein, and the MPC manufactured at treatment level 4 contains on average 0.064 mol Na per 100 g protein (calculations

shown in Appendix N). Dry powder protein and sodium values used in these calculations were obtained by least squares means from the analyses titled "General Linear Model: Na versus block, treatment" in Appendix N.

5.4. Moisture analysis

The moisture content of dry MPC powder, as determined according to AOAC 925.23, is shown in Table 7. The statistical analysis of the moisture content of dry MPCs was conducted using the GLM command in Minitab according to section 4.5.4. There was no significant difference in moisture content of MPC ($p = 0.107$). Therefore it can be concluded that the differences in solubility that were observed between the treatment levels cannot be attributed to differences in MPC moisture content. Minitab output from Tukey's simultaneous comparisons is located in Appendix O.

Table 7: Moisture content of dry MPCs; Treatment averaged across all blocks, different superscripts denote moisture results that are statistically significantly different from each other according to Tukey's simultaneous tests.

Sample	Treatment (mM NaCl in DF H_2O)	Moisture (%)
MPC	0	7.13[a]
MPC	50	6.04[a]
MPC	100	5.79[a]
MPC	150	6.56[a]

5.5. Powder Particle Size

5.5.1. Particle Size Distribution of Dry MPC Powder

The particle size of dry MPC powder, as determined by Coulter LS230 with dry powder module, is shown below (Table 22). To determine the treatment effect on the size of the of powder particles, statistical analysis of d_{90} was conducted using the GLM command in Minitab according to section 4.5.4.

At $\alpha = 0.01$, there was no statistically significant difference in particle size (d_{90}) due to the effect of NaCl treatment ($p = 0.798$) or mean particle size ($p = 0.878$) due to the effect of NaCl treatment. For complete detail, the full particle size distributions of MPC powders for each treatment level averaged across all blocks are displayed below (Figure 42, Figure 43, Figure 44, Figure 45).

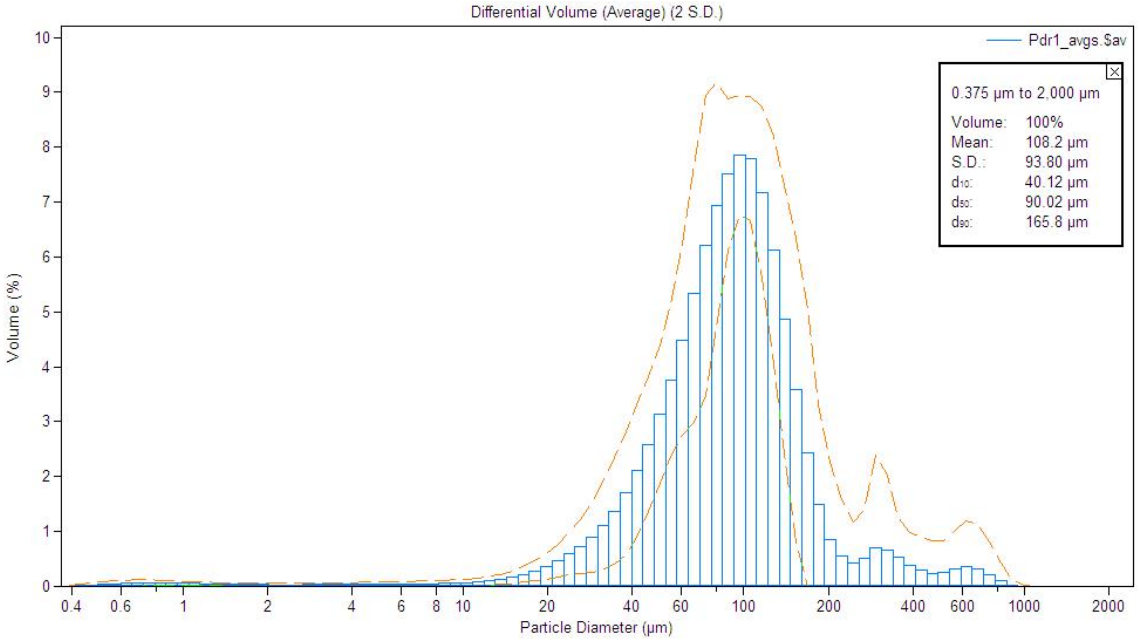

Figure 42: Average particle size distribution of MPCs manufactured with no NaCl in DF water (treatment level 1) across all blocks.

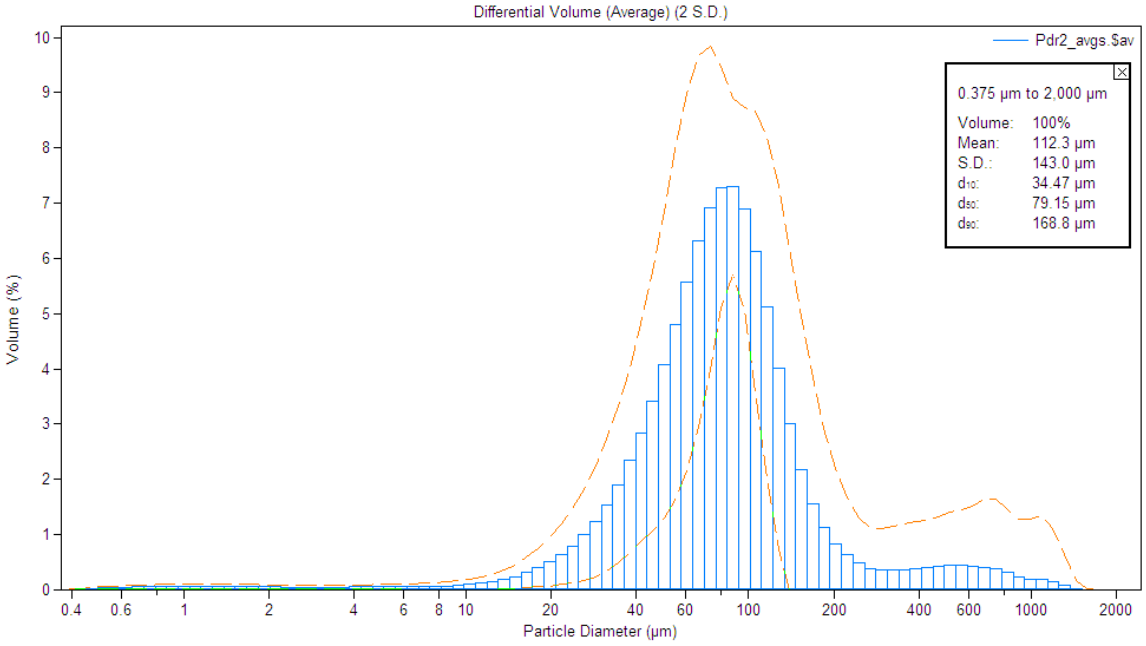

Figure 43: Average particle size distribution of MPCs manufactured with 50 mM NaCl in DF water (treatment level 2) across all blocks.

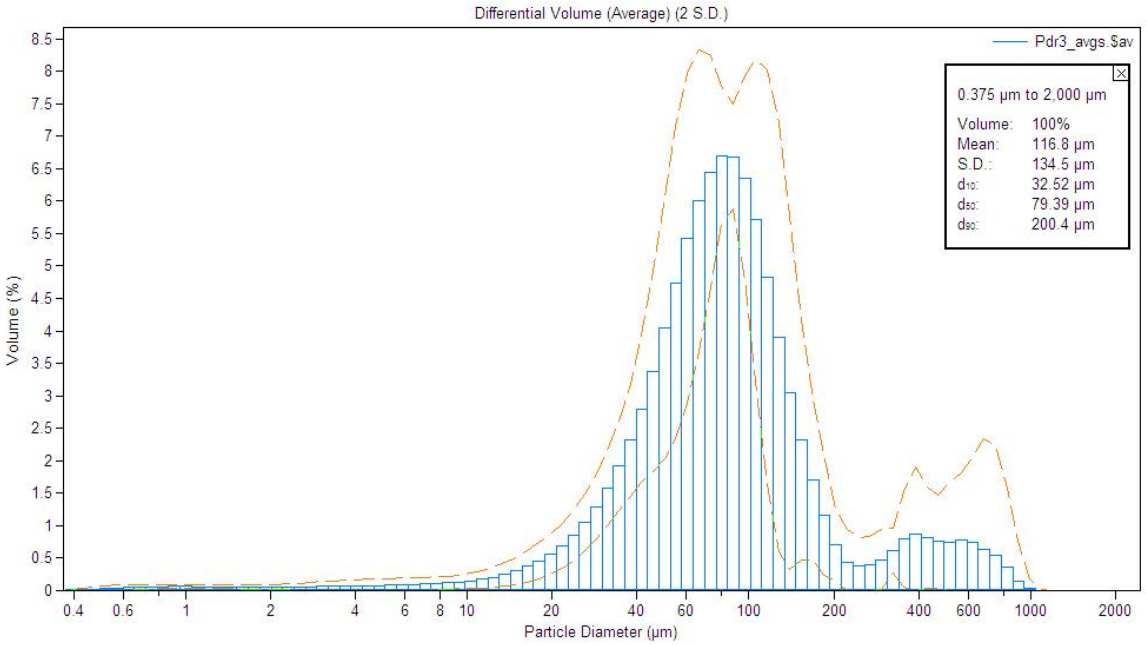

Figure 44: Average particle size distribution of MPCs manufactured with 100 mM NaCl in DF water (treatment level 3) across all blocks.

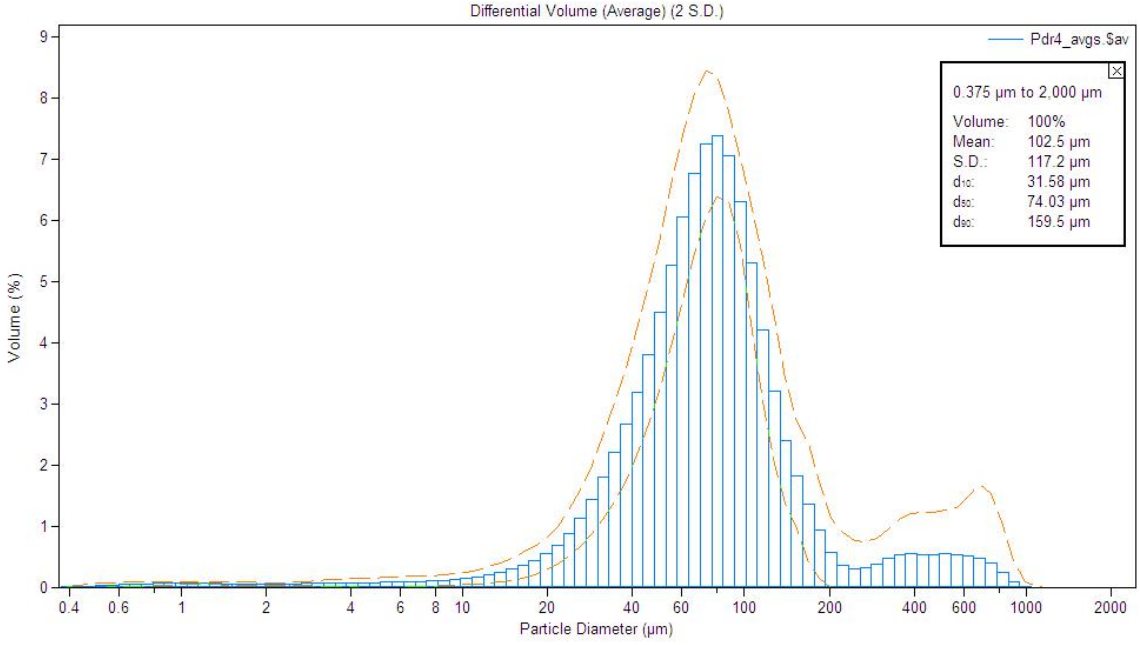

Figure 45: Average particle size distribution of MPCs manufactured with 150 mM NaCl in DF water (treatment level 4) across all blocks.

The statistical analysis was unable to detect a difference in the dry MPC particle sizes (as expressed by both the d_{90} and mean) between MPCs manufactured at different treatment levels. This result suggests that NaCl addition to DF water may not impact dry MPC particle size. Currently, no other study has attempted to detect a difference in dry MPC particle size due to addition of sodium.

5.5.2. Particle Size Distribution of MPC Powder During Reconstitution

The particle size of MPC during reconstitution, as determined by Coulter LS230 with fluid module, is shown in Appendix P. To determine the treatment effect on the size of of particles, statistical analysis of d_{90} and mean particle size were conducted using the GLM command in Minitab according to section 4.5.4. At $\alpha = 0.01$, there was no statistically significant difference in d_{90} ($p = 0.798$) or mean particle size ($p = 0.878$) due to the effect of NaCl treatment.

The full particle size distributions of MPC powders for each treatment level averaged across all blocks is also displayed below (Figure 46, Figure 47, Figure 48, Figure 49).

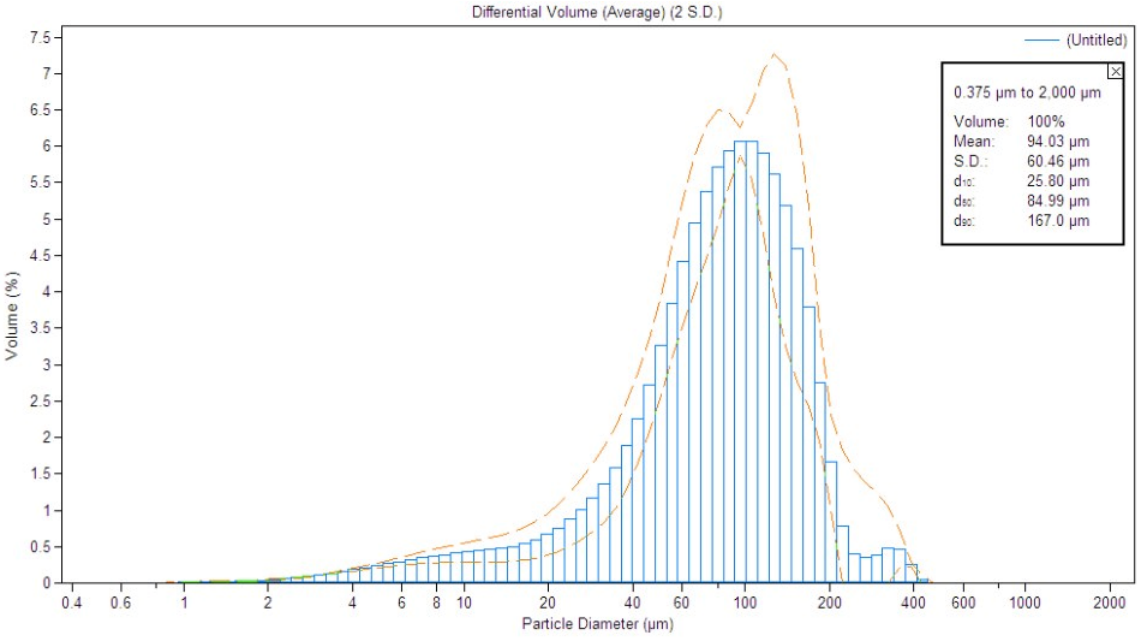

Figure 46: Average particle size distribution of particles during reconstitution resulting from the manufacture of MPC utilizing no NaCl in DF water (treatment level 2) across all blocks

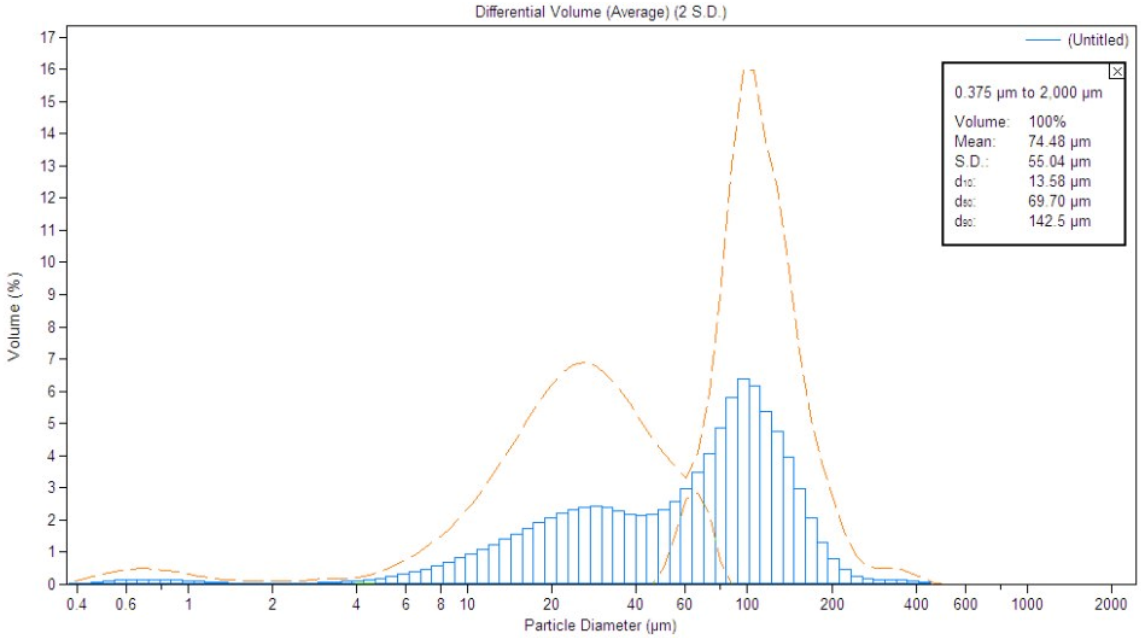

Figure 47: Average particle size distribution of particles during reconstitution resulting from the manufacture of MPC utilizing 50 mM NaCl in DF water (treatment level 1) across all blocks

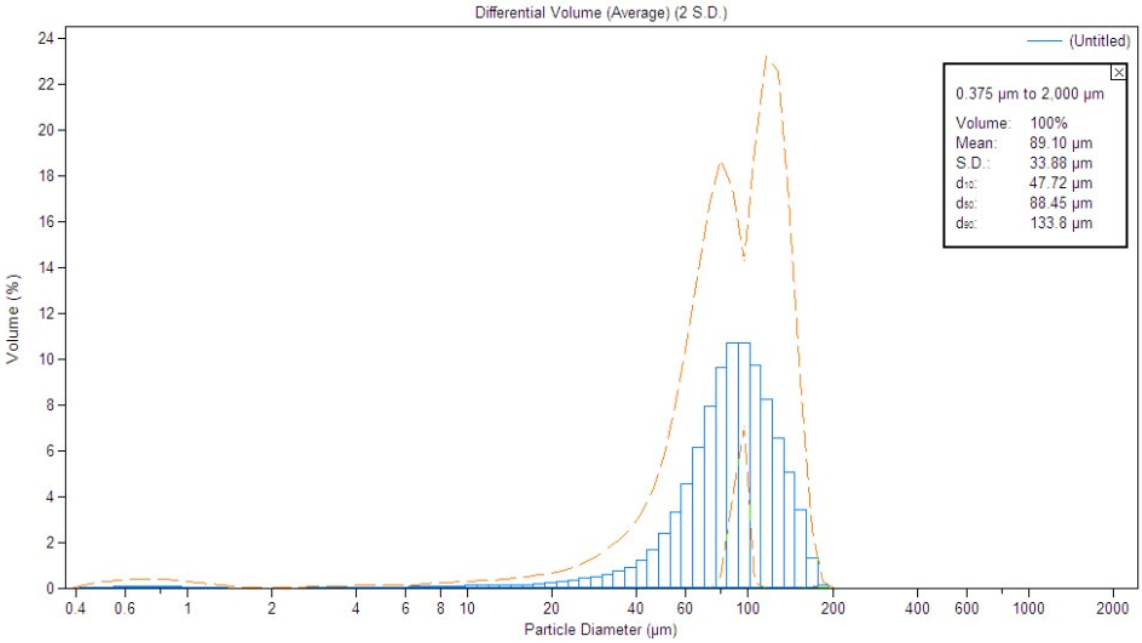

Figure 48: Average particle size distribution of particles during reconstitution resulting from the manufacture of MPC utilizing 100 mM NaCl in DF water (treatment level 1) across all blocks

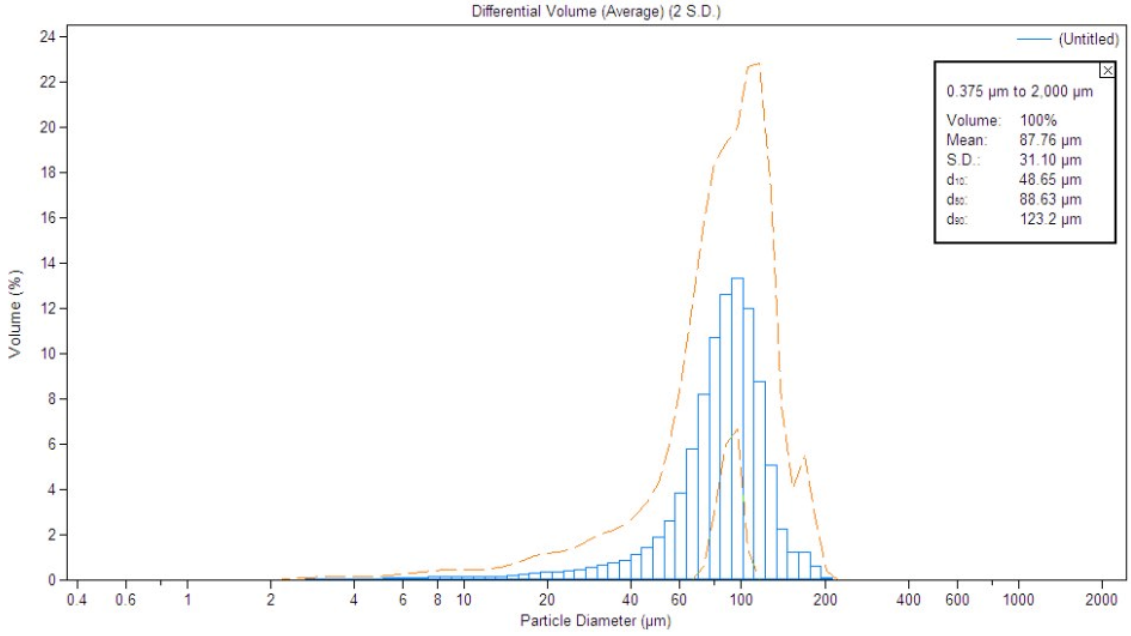

Figure 49: Average particle size distribution of particles during reconstitution resulting from the manufacture of MPC utilizing 150 mM NaCl in DF water (treatment level 1) across all blocks

It is generally expected that, as the solubility of dairy powders increases, particle size will trend closer to that of native micelle size in milk. This study did not detect a difference in either d_{90} or mean particle size between the treatment groups. It is possible that powders were not reconstituted for a long enough time during this experiment to observe more significant decreases in particle size upon reconstitution or that the number of replicates was too small to detect a difference. It is also possible that observed increases in solubility are due to changes in particle structure, not necessarily a significant decrease in particle size. This possibility will be discussed in section 5.6.

It is also possible that analysis of d_{90} or mean particle size, by themselves, do not accurately describe changes in particle size distribution that could be occurring as a function of the treatment. This is illustrated by a scatterplot of particle size averaged across blocks vs. NaCl treatment (Figure 50). The scatterplot shows that particle size distribution appears to narrow as NaCl treatment is increased. To assist in the analysis of MPC particle size distribution upon reconstitution, an analysis of the mid80 range (d_{90}-d_{10}) was conducted using the GLM command in Minitab according to section 4.5.4. At $\alpha = 0.01$, there was no statistically significant difference in mid80 range ($p = 0.017$) due to the effect of NaCl treatment. Therefore, this study cannot conclude that significant changes in particle size occurred in MPC during reconstitution as a function of NaCl treatment up to 150 mM NaCl in DF water, the highest treatment level tested.

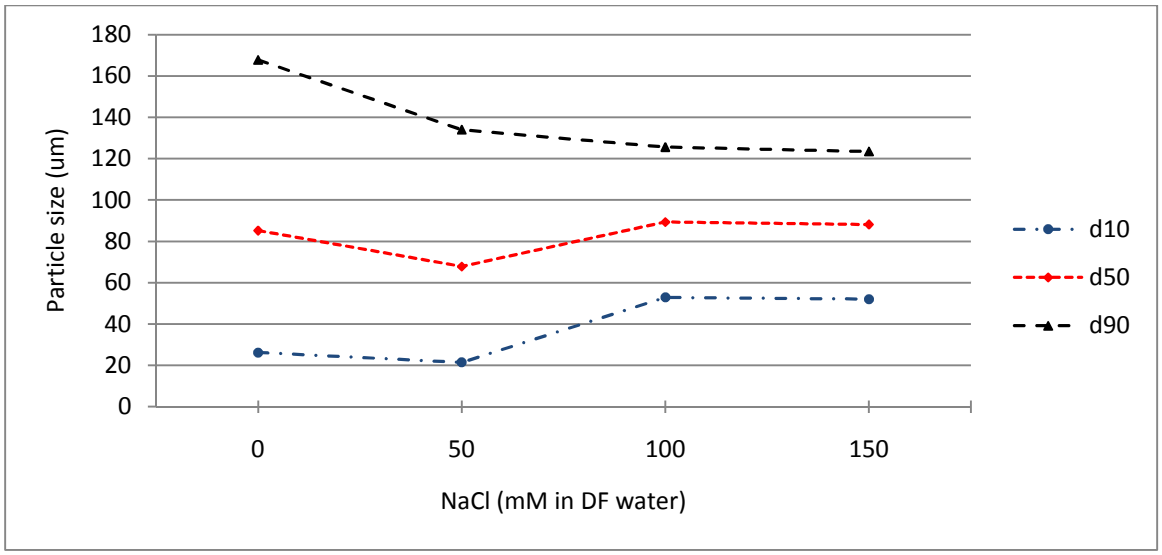

Figure 50: Scatterplot of particle size averaged across blocks vs. NaCl (mM in DF water)

Further analysis was conducted to investigate possible changes in particle size of MPC during reconstitution. An analysis of the right tail of the particle size distribution, defined as (d_{90}-d_{50}), was conducted using the GLM command in Minitab as described in section 4.5.4. At $\alpha = 0.01$, there was a statistically significant difference in (d_{90}-d_{50}) ($p = 0.008$) due to the effect of NaCl treatment (Table 8).

Despite the GLM finding a significant difference between the treatment levels, Tukey's method of pairwise comparisons did not yield statistically significant differences between means. This may be due to differences in the nature of the two tests; the GLM is a slightly more powerful method than the method of Tukey's pairwise comparisons. The significant F-test from the GLM indicates that (d_{90}-d_{50}) is significantly different between the treatments. Since the Tukey intervals cannot detect which treatments are significantly different, we interpret that a significant difference exists between 0 mM NaCl and 150 mM NaCl, the lowest and highest treatments.

Table 8: Average particle size distribution (d_{90}-d_{50}) of particles during reconstitution resulting from the manufacture of MPC; treatment averaged across all blocks; different superscripts denote particle size distribution (d_{90}-d_{10}) results that are statistically significantly different from each other according to Tukey's simultaneous tests; although GLM results were significant (p = 0.008), Tukey's simultaneous tests did not find significant differences between the means.

Treatment (mM NaCl in DF H_2O)	Particle Size (d90-d50)
0	82.6[a]
50	66.2[a]
100	36.2[a]
150	35.3[a]

5.6. Confocal laser scanning microscopy

CLSM of reconstituted MPCS were taken according to the method outlined in Section 4.5.3. Figure 51 shows differences in MPC particle microstructure between MPC manufactured with no NaCl (top left), 50 mM (top right), 100 mM (bottom left), and 150 mM (bottom right) NaCl incorporated into DF water. Upon reconstitution, MPC particles manufactured without NaCl addition to DF water appeared to contain a structural network of greater density than MPC particles manufactured with 50 mM or 100 mM NaCl incorporated into DF water. As NaCl treatment level increases, the particle structural network appeared to become more porous, as can be seen in Figure 51.

If an increase in porosity is observed, then a decrease in particle density upon reconstitution should also be observed; however, no particle density measurements were conducted. It is recommended that future research further explore possible connections

between the manufacture of MPC utilizing the addition of NaCl to DF water, particle porosity, and changes in particle density.

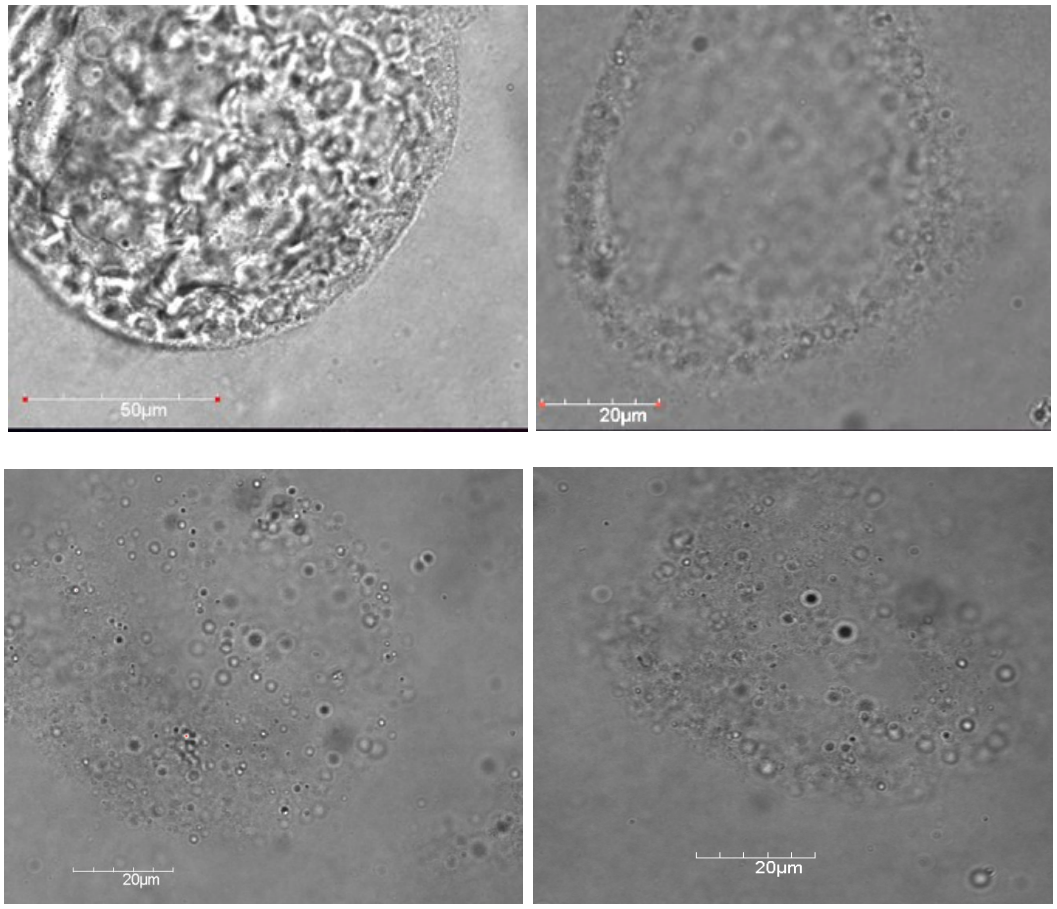

Figure 51: (Top Left) Confocal micrograph of MPC manufactured at block 3, treatment level 1 (scale = 50 µm). (Top Right) Confocal micrograph of MPC manufactured at block 2, treatment level 2 (scale = 50 µm). (Bottom Left) Confocal micrograph of MPC manufactured at block 3, treatment level 3 (scale length = 20 µm). (Bottom Right) Confocal micrograph of MPC manufactured at block 3, treatment level 4 (scale = 20 µm).

It was previously observed that the microstructure of fresh MPC85 powders after rehydration for 10 min. at 24 °C displayed a "skin-like structure" on their surfaces (Mimouni et al., 2010).The micrographs in Figure 51, particularly the control (top left), and 50 mM NaCl treatment (top right) support that observation. In the micrograph

containing an MPC manufactured without NaCl in DF water (top left), there is a clear distinction between the particle material and the surrounding space. In contrast, in the MPC particle manufactured with 50 mM NaCl in DF water (top right), and 100 mM NaCl in DF water (bottom left), and 150 mM DF water (bottom right), the separation between the particle material and the surrounding space is less distinct.

It was also previously observed that the microstructure of hydrated MPC85 powder particles, under conditions of mixing at 24 °C for 80 min., became relatively porous, and these cavities are large enough to enable water to penetrate the particle interior (Figure 52, bottom right) (Mimouni et al., 2010). This observation was made by comparing MPC85 (of which no data pertaining to mineral content or solubility was reported) at different reconstitution times and temperatures, and at different storage times, using scanning electron microscopy. Together, the images captured by Mimouni et al. (2010) demonstrate a similar progression as the one demonstrated in Figure 51, from a particle exhibiting a skin-like structure upon rehydration (Figure 52, top left), to a particle with an interior structure appearing more porous in nature (Figure 52, top right), to a particle that is breaking apart (Figure 52, bottom left). Though solubility data was not reported, MPCs stored 2 months at 20 °C (Figure 52, top left) should exhibit poorer solubility, according to Havea et al (2006) and Carr et al. (2002), than freshly made MPC reconstituted for 10 min. at 24 °C (Figure 52, top right) and 80 min (Figure 52, bottom left). It was concluded that the dissolution of powder particles occurred by a gradual process, in which the external particle surface and internal surfaces (when exposed by breaches in the external surface) were eroded by the penetration of water throughout the particle (Mimouni et al., 2010).

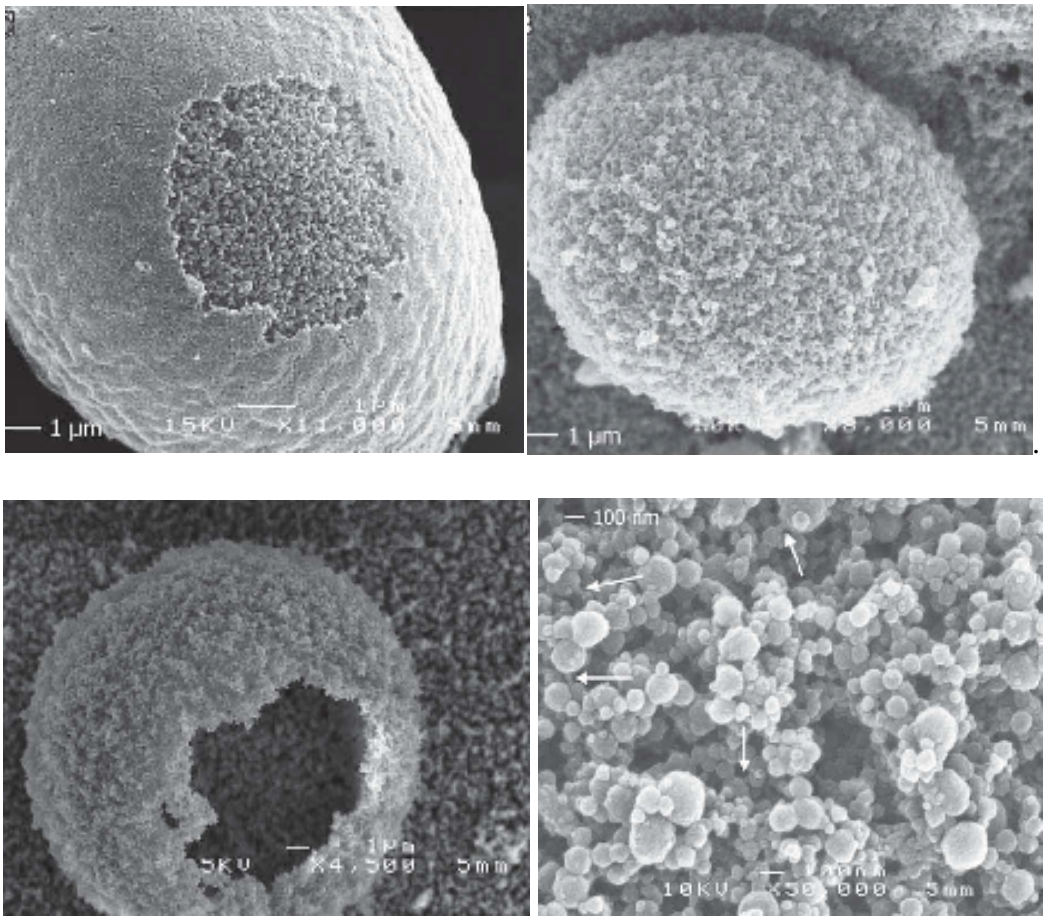

Figure 52: Field emission scanning electron micrographs of MPC after rehydration, all scales = 1 µm; (Top left) aged milk protein concentrate after 2 months storage at 24 °C hydrated for 10 min. at 24 °C (scale = 1 µm), (Top right) freshly made MPC hydrated for 10 min. at 24 °C, (Bottom left) freshly made MPC hydrated for 80 min. at 24 °C, (Bottom right) details of the surface of hydrated powder particles, white arrows indicate presence of intermicellar bridges. Images captured by Momouni et al (2010)

Taken together, there exist observed similarities between the images in Figure 51 and those in Figure 52; both sets of images depict MPC particles with a "skin-like layer", followed by what appears to be a gradual dissolution (both from the exterior and interior) of the MPC particle, and both demonstrate an apparent change in the porosity of MPC. If the mechanisms of dissolution demonstrated in Figure 51 and Figure 52 are indeed

similar to each other, it would indicate that the addition of NaCl into DF water has accelerated the mechanism by which MPC dissolves. In other words, the forces which bridge micellar networks (described as intermicellar contacts and short bridges by Mimouni (2010)) may have been weakened by the addition of NaCl into DF water ,or that forces existed which counteracted the forces which favored the bridging of micellar networks. This assumes that that these intermicellar contacts and short bridges are a significant inhibitor of MPC dissolution, but the role of these physiochemical interactions has not yet been isolated or determined.

The cause of this acceleration in the dissolution mechanism it is not known. Testing if the speed of the dissolution mechanism heavily depends upon the ionic strength of the MPC as it is reconstituted would require a measurement of important minerals during the reconstitution process, including determining the ratio of ionic phase minerals to colloidal phase minerals. If, however, the addition of NaCl to DF water during the manufacture significantly alters the mechanism by which the product is converted from liquid retentate to dry form, then high-resolution examination of the MPCs in dry form (utilizing TEM or SEM techniques) may indicate differences in the arrangement of material on the powder surface. To date, although TEM has been used to show changes in MPC morphology under storage conditions (Carr et al., 2002), direct evidence of physical differences (in dry form) between MPC manufactured with mineral addition and MPC manufactured without have not been reported. Particle size analysis of dry MPC, discussed in section 5.5.2, failed to find significant differences in dry MPC particle size due to effect of NaCl treatment.

In this study, the utilization of FG and NR in CLSM allows some observations to be made concerning the distributions of lipids and proteins on the surface of MPC powder particles. It was observed that MPC powders manufactured without the addition of NaCl into DF water had a high incidence of lipid material on MPC particle surfaces; and there appeared to be only small amounts of lipid material present that were not bound to particle surfaces. These observations are illustrated by Figure 53, which shows MPC powder particles manufactured without addition of NaCl into DF water. Both the intensity and concentration of lipid material on the surface of powder particles in Figure 53 appear different than those in Figure 54, which show MPC powder particles manufactured at treatment level 2 (top left and top right) and treatment level 3 (bottom left and bottom right).

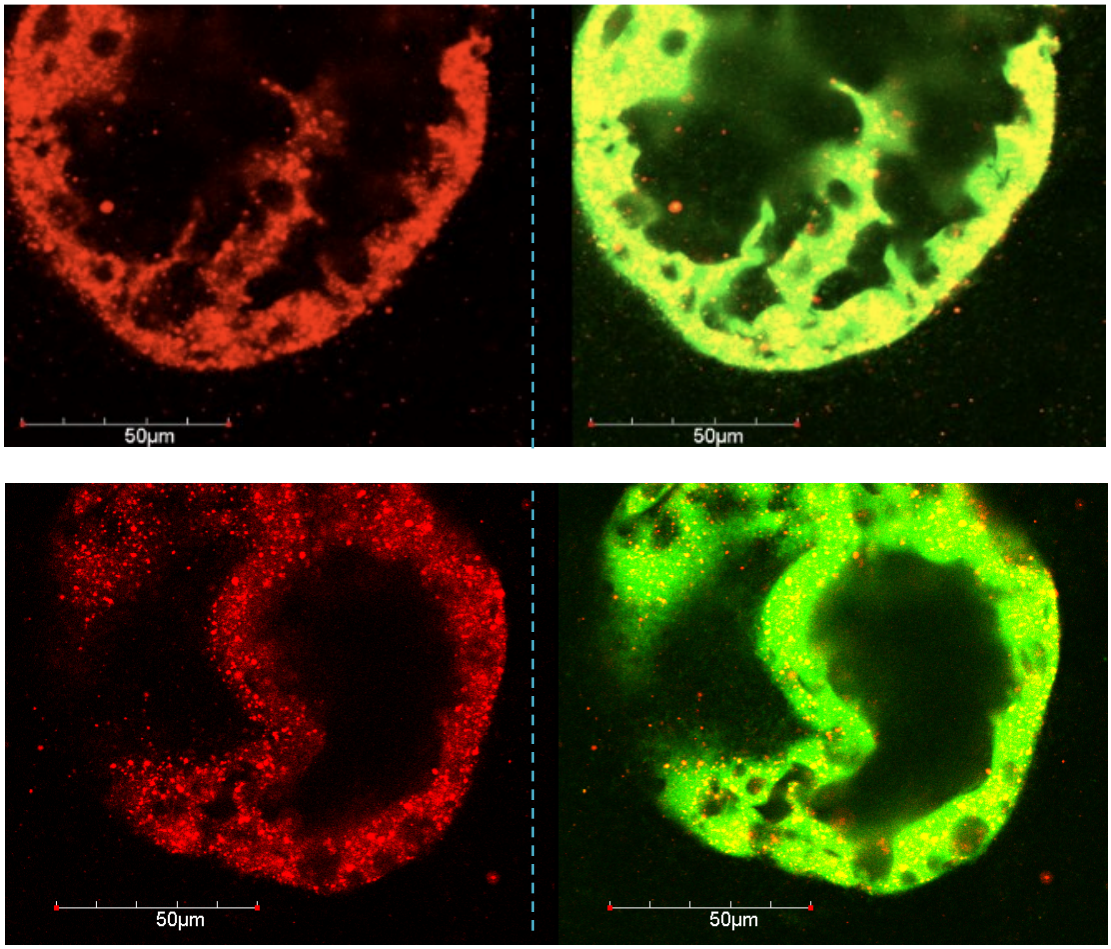

Figure 53: Top left and top right: confocal micrograph of powder manufactured at block 3, treatment level 1 (scale = 50 µm); (Top left) NR excitation only, (Top right) excitation of both FG and NR; Bottom left and bottom right: confocal micrograph of MPC manufactured at block 2, treatment level 1 (scale = 50 µm); (Left) NR excitation only, (Right) excitation of both FG and NR

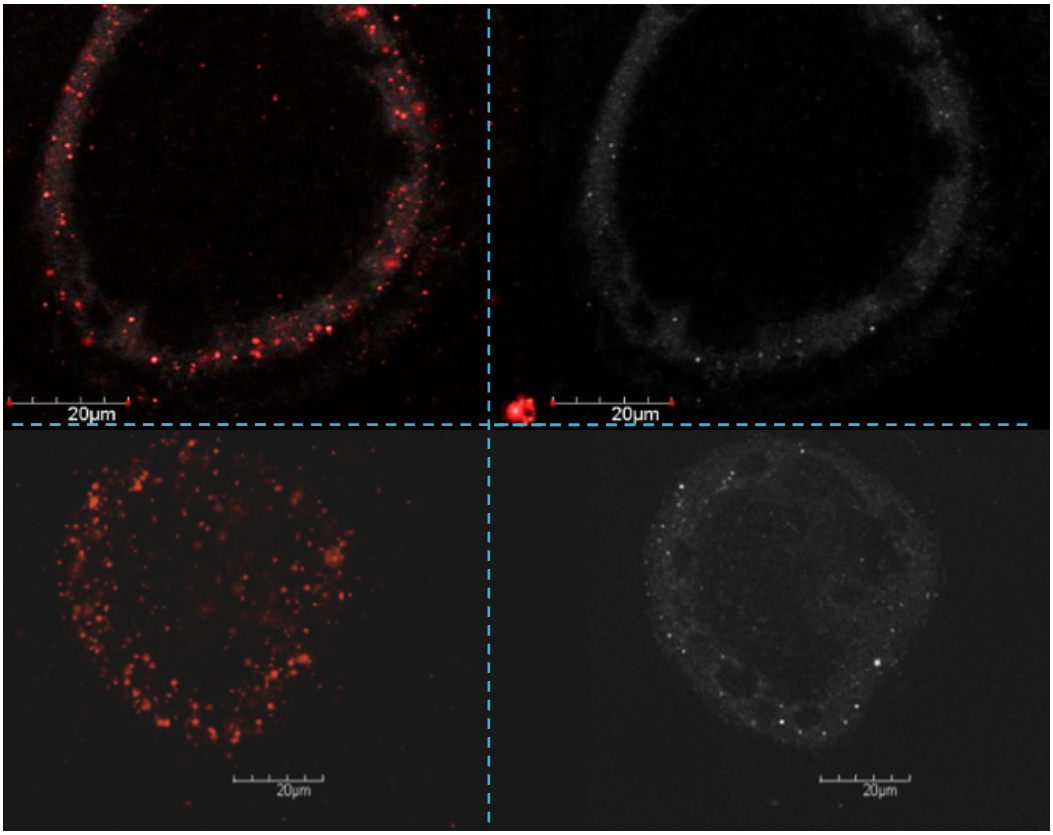

Figure 54: Top left and top right: confocal micrograph of MPC manufactured at block 2, treatment level 2 (scale = 20 µm); (Left) FG and NR excitation, (Right) excitation of only FG; Bottom left and bottom right: confocal micrograph of MPC manufactured at block 3, treatment level 3 (scale = 20 µm); (Left) FG and NR excitation, (Right) excitation of only FG

A comparison of three-dimensional confocal micrographs (Figure 55) indicate that MPC manufactured with 150 mM NaCl in DF water appears to contain a very high incidence of lipid material that is not bound to any powder particle (left), in comparison with MPC manufactured without NaCl incorporated into DF water (right), in which the majority of lipid material appears to be associated with powder particles.

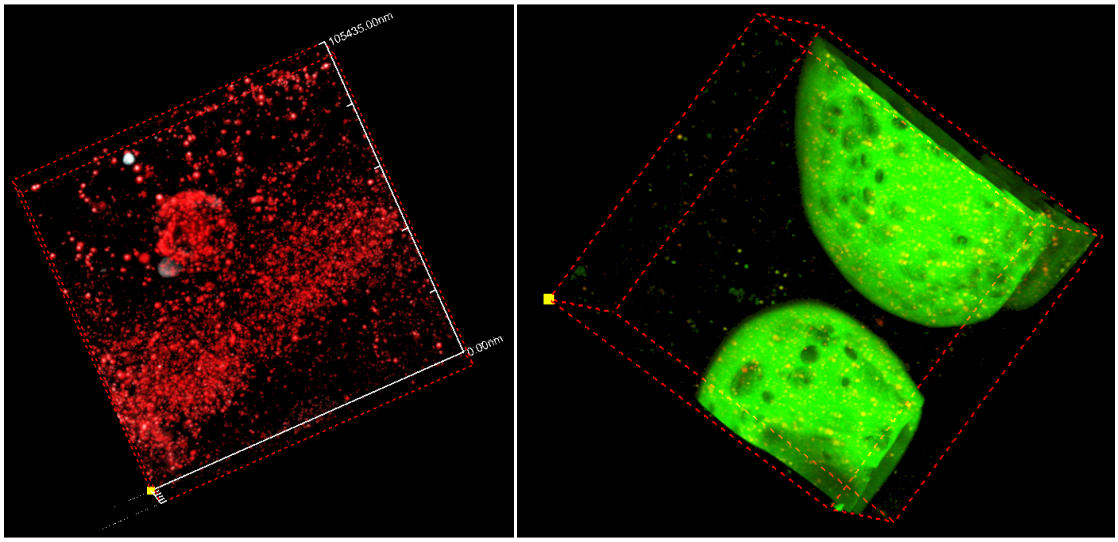

Figure 55: Confocal micrographs of MPC manufactured at block 2, treatment level 4, NR and FG excitation (left), and block 1 treatment level 1, NR and FG excitation (right) (length and width of cross sections in both images = 105.44 µm)

Milk fat is believed to play a role in the behavior of powder during reconstitution (Farkye, 2006). It was previously observed in milk powder that the amount of free fat (defined as the fraction of total fat that is not protected by protein film, but is instead present as "pools" or "patches" instead of as globules on the powder particle surface) (Pisecky, 1997) was inversely related to the particle size (Buma, 1971). According to Zayas (1997), in high powder protein foods, fat binding is influenced by the size of the powder particles. However, these observations have not been tested in an MPC system.

There is some information relating the packed bulk density of milk powders with fat content; a study by Tuohy (1989) suggested that increasing fat content of milk powder could yield a milk powder with decreased bulk density. If applicable to MPC, this would indicate that a decrease in the fat content of MPC could result in an improvement in the poor bulk density typically exhibited by these powders, but there are currently no studies to suggest these same assumptions are applicable. In addition, there is little to no information in the scientific literature on the role of milk fat on the dispersability, flowability, or bulk density of milk powders (Farkye, 2006), much less the role of milk fat on these properties in MPC.

In dairy powders, it can be assumed that fat may exist in one of four states:: as surface fat (present as patches on the surface of particles), outer layer fat (consisting of fat globules that nearly touch the particle surface), capillary fat (fat globules that come into contact with the surface of micropores), and dissolution fat (fat globules that contact any openings after the particle's outer layer has been dissolved) (Pisecky, 1997). Regardless of state, nonpolar side-chasins of protein molecules are the primary sites of lipid protein interactions; thus hydrophobic and, potentially water-insoluble, proteins should have a higher capacity to bind fat (Zayas, 1997).

The use of one or more hydrophobic probes may aid in determining if MPC manufactured without NaCl addition to DF water exhibits increased hydrophobicity; if so then this would lend additional evidence to the hypothesis that there are increased lipid-protein interactions occurring in MPC manufactured without the addition of NaCl to retentate.

6. CONCLUSIONS AND SIGNIFICANCE

The main objective of this thesis was to test a manufacturing process utilizing the addition of sodium chloride to diafiltration water, which ultimately yields a spray-dried milk protein concentrate powder with improved solubility over a powder which is not manufactured with the addition of sodium chloride to diafiltration water.

Milk protein concentrate manufactured utilizing the addition of sodium chloride to diafiltration water showed improved solubility under a variety of solubilizing conditions. For example, it was observed that under conditions of reconstitution for 1h at 23 °C ± 1 °C, mean MPC solubility was improved from 59.81 % (when no NaCl was incorporated into DF water) to 96.52 % (when 150 mM NaCl was incorporated into DF water). Under these testing conditions, significant increases in mean solubility were observed at every NaCl treatment level tested. In addition, it was observed under conditions of reconstitution for 30 min at 30 °C using an overhead stirrer, the addition of 150 mM NaCl into DF water improved mean solubility to 96.2 %, a significant increase from the control level's mean solubility of 46.1 %. In each solubility test performed, the solubility of milk protein concentrate manufactured utilizing sodium chloride in DF water was significantly higher than that of the control. These results demonstrate that milk protein concentrate manufactured by this process was significantly more soluble in water than milk protein concentrate that had been manufactured without such NaCl addition. Analysis of retentate products obtained by ultrafiltration and diafiltration, as well as of the final powders, showed significant increases in sodium content in both retentate and powder as sodium chloride treatment level increased

Analysis of particle size revealed no significant differences in dry powder particle size or particle size upon reconstitution due to sodium chloride treatment level. However, using light microscopy, some differences in rehydrated particle structure could be observed between treatment levels. These differences may partially explain differences in milk protein concentrate solubility between treatment levels.

This thesis project provides a preliminary foundation for future studies investigating the effect of sodium chloride addition on particle structure, and the cause of such increases in solubility as sodium chloride treatment level increased. These insights may assist milk protein concentrate manufacturers in producing powders with consistently high water solubility.

7. DIRECTIONS FOR FUTURE RESEARCH

Additional research into the relationship between milk protein concentrate manufacturing methods involving the addition of sodium chloride into the diafiltration water and its impact on the solubility of the final powder may broaden our understanding of the mechanism by which sodium addition yields a more highly soluble milk protein concentrate powder. Possible research directions are as follows:

1. Investigate the location and state of added minerals by quantifying the concentrations of minerals bound to proteins versus the concentrations of minerals existing as ions upon reconstitution
2. Isolate the effect of sodium addition on the solubility of the final powder from the effect of calcium depletion
3. Further investigate the relationship between mineral salt addition during the MPC manufacture process and changes in particle size and structure that occur during reconstitution of the powder
4. Determine changes in protein conformation and hydrophobic interactions that occur as MPC retentate is gradually replenished with sodium
5. Perform MPC manufacturing trials as described in this thesis utilizing other mineral salts which dissociate to yield monovalent or divalent cations, such as potassium chloride or calcium chloride

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Appendix A: Calculation of Statistical Power

$$df = a(n-1)$$

$$F_1 = F_{(a-1),df,(1-\alpha)}$$

$$\lambda = \left(\frac{d^2}{2}\right)\left(\frac{n}{\sigma^2}\right)$$

$$F_2 = F^{-1}_{(a-1),df,0.05,\lambda}$$

$$Power = 1 - F_2$$

Figure 56: Equations utilized to calculate statistical power when "Power and Sample Size for One-Way Anova" command is invoked in Minitab v.15.1 (Anonymous, 2010d), where df = degrees of freedom, a = number of factor levels, n = sample size per factor level, d = maximum difference between means, σ = overall standard deviation, and α = significance level

Appendix B: Temperature and pH of Products Obtained by MPC Manufacture

Table 9: pH and temperature of each product obtained from MPC manufacture

Block	Treatment	Sample	pH	Temperature (°C)
1	1	SM	6.85	5.1
1	1	UF	6.75	17.2
1	1	DF1	6.81	23.0
1	1	DF2	6.87	27.4
1	1	DF3	7.14	28.2
1	2	SM	6.85	5.3
1	2	UF	6.77	19.0
1	2	DF1	6.81	27.4
1	2	DF2	6.87	29.0
1	2	DF3	7.14	30.7
1	3	SM	6.84	8.3
1	3	UF	6.66	18.3
1	3	DF1	6.75	27.1
1	3	DF2	6.95	31.1
1	3	DF3	7.15	30.4
1	4	SM	6.84	5.0
1	4	UF	6.66	19.1
1	4	DF1	6.75	27.1
1	4	DF2	6.95	31.1
1	4	DF3	7.15	30.4
2	1	SM	6.87	7.1
2	1	UF	6.71	18.4
2	1	DF1	6.83	25.3
2	1	DF2	6.98	29.8
2	1	DF3	7.09	30.3
2	2	SM	6.87	7.2
2	2	UF	6.72	19.1
2	2	DF1	6.84	25.7
2	2	DF2	6.97	31.1
2	2	DF3	7.16	31.0
2	3	SM	6.84	5.20
2	3	UF	6.68	22.5
2	3	DF1	6.76	29.4
2	3	DF2	6.97	31.1
2	3	DF3	7.18	31.5

Table 7; continued

Block	Treatment	Sample	pH	Temperature (°C)
2	4	SM	6.83	4.90
2	4	UF	6.67	20.9
2	4	DF1	6.78	26.7
2	4	DF2	6.97	29.9
2	4	DF3	7.23	29.5
3	1	SM	6.85	5.0
3	1	UF	6.77	21.8
3	1	DF1	6.84	26.3
3	1	DF2	6.95	28.8
3	1	DF3	7.10	30.5
3	2	SM	6.87	7.4
3	2	UF	6.75	19.1
3	2	DF1	6.87	25.7
3	2	DF2	7.03	31.2
3	2	DF3	7.22	29.9
3	3	SM	6.90	5.5
3	3	UF	6.74	20.7
3	3	DF1	6.84	26.9
3	3	DF2	7.04	30.4
3	3	DF3	7.31	29.4
3	4	SM	6.84	4.8
3	4	UF	6.76	20.2
3	4	DF1	6.87	24.9
3	4	DF2	7.09	28.2
3	4	DF3	7.27	33.6

Statistical Analysis to Determine if pH differed significantly due to Treatment, sliced by Process

The Mixed Procedure

Model Information	
Data Set	WORK.PH_TEMP
Dependent Variable	pH
Covariance Structure	Variance Components
Subject Effect	block*treatment
Estimation Method	REML
Residual Variance Method	Parameter
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	**Levels**	**Values**
process	5	DF1 DF2 DF3 SM UF
sample	12	1 2 3 4 5 6 7 8 9 10 11 12
block	3	1 2 3
treatment	4	1 2 3 4

Dimensions	
Covariance Parameters	3
Columns in X	30
Columns in Z	15
Subjects	1
Max Obs Per Subject	60

Number of Observations	
Number of Observations Read	60
Number of Observations Used	60
Number of Observations Not Used	0

Iteration History			
Iteration	**Evaluations**	**-2 Res Log Like**	**Criterion**
0	1	-107.13146999	
1	1	-130.11163270	0.00000000

Convergence criteria met.

Estimated R Matrix for block*treatment 1 1					
Row	**Col1**	**Col2**	**Col3**	**Col4**	**Col5**
1	0.000994				
2		0.000994			
3			0.000994		
4				0.000994	
5					0.000994

Estimated R Correlation Matrix for block*treatment 1 1					
Row	**Col1**	**Col2**	**Col3**	**Col4**	**Col5**
1	1.0000				
2		1.0000			
3			1.0000		
4				1.0000	
5					1.0000

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
block		0.001100
block*treatment		0.000228
process	block*treatment	0.000994

Fit Statistics	
-2 Res Log Likelihood	-130.1
AIC (smaller is better)	-124.1
AICC (smaller is better)	-123.4
BIC (smaller is better)	-126.8

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
treatment	3	6	0.62	0.6285
process	4	32	375.19	<.0001
process*treatment	12	32	3.49	0.0023

Least Squares Means							
Effect	**process**	**treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
process*treatment	DF1	1	6.8267	0.02782	32	245.40	<.0001
process*treatment	DF1	2	6.8400	0.02782	32	245.88	<.0001
process*treatment	DF1	3	6.7833	0.02782	32	243.84	<.0001
process*treatment	DF1	4	6.8000	0.02782	32	244.44	<.0001

Least Squares Means							
Effect	**process**	**treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
process*treatment	DF2	1	6.9333	0.02782	32	249.23	<.0001
process*treatment	DF2	2	6.9567	0.02782	32	250.07	<.0001
process*treatment	DF2	3	6.9867	0.02782	32	251.15	<.0001
process*treatment	DF2	4	7.0033	0.02782	32	251.75	<.0001
process*treatment	DF3	1	7.1100	0.02782	32	255.58	<.0001
process*treatment	DF3	2	7.1733	0.02782	32	257.86	<.0001
process*treatment	DF3	3	7.2133	0.02782	32	259.30	<.0001
process*treatment	DF3	4	7.2167	0.02782	32	259.42	<.0001
process*treatment	SM	1	6.8567	0.02782	32	246.48	<.0001
process*treatment	SM	2	6.8633	0.02782	32	246.72	<.0001
process*treatment	SM	3	6.8600	0.02782	32	246.60	<.0001
process*treatment	SM	4	6.8367	0.02782	32	245.76	<.0001
process*treatment	UF	1	6.7433	0.02782	32	242.40	<.0001
process*treatment	UF	2	6.7467	0.02782	32	242.52	<.0001
process*treatment	UF	3	6.6933	0.02782	32	240.60	<.0001
process*treatment	UF	4	6.6967	0.02782	32	240.72	<.0001

Tests of Effect Slices					
Effect	**process**	**Num DF**	**Den DF**	**F Value**	**Pr > F**
process*treatment	DF1	3	32	1.61	0.2069
process*treatment	DF2	3	32	2.38	0.0877
process*treatment	DF3	3	32	6.05	0.0022
process*treatment	SM	3	32	0.35	0.7876
process*treatment	UF	3	32	2.06	0.1258

Statistical Analysis to Determine if Temperature differed significantly due to Treatment

The Mixed Procedure

Model Information	
Data Set	WORK.PH_TEMP
Dependent Variable	Temp
Covariance Structures	Variance Components, Autoregressive
Subject Effect	block*treatment
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	**Levels**	**Values**
process	5	DF1 DF2 DF3 SM UF
sample	12	1 2 3 4 5 6 7 8 9 10 11 12
block	3	1 2 3
treatment	4	1 2 3 4

Dimensions	
Covariance Parameters	4
Columns in X	30
Columns in Z	15
Subjects	1
Max Obs Per Subject	60

Number of Observations	
Number of Observations Read	60
Number of Observations Used	60
Number of Observations Not Used	0

Iteration History			
Iteration	**Evaluations**	**-2 Res Log Like**	**Criterion**
0	1	159.81993823	
1	3	156.15140861	0.00358441
2	2	155.99373285	0.00004255
3	1	155.99196528	0.00000001

Convergence criteria met.

Estimated R Matrix for block*treatment 1 1					
Row	**Col1**	**Col2**	**Col3**	**Col4**	**Col5**
1	1.4933	-0.5629	0.2122	-0.07997	0.03014
2	-0.5629	1.4933	-0.5629	0.2122	-0.07997
3	0.2122	-0.5629	1.4933	-0.5629	0.2122
4	-0.07997	0.2122	-0.5629	1.4933	-0.5629
5	0.03014	-0.07997	0.2122	-0.5629	1.4933

Estimated R Correlation Matrix for block*treatment 1 1					
Row	**Col1**	**Col2**	**Col3**	**Col4**	**Col5**
1	1.0000	-0.3769	0.1421	-0.05355	0.02019
2	-0.3769	1.0000	-0.3769	0.1421	-0.05355
3	0.1421	-0.3769	1.0000	-0.3769	0.1421

Estimated R Correlation Matrix for block*treatment 1 1					
Row	**Col1**	**Col2**	**Col3**	**Col4**	**Col5**
4	-0.05355	0.1421	-0.3769	1.0000	-0.3769
5	0.02019	-0.05355	0.1421	-0.3769	1.0000

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
block		0.03548
block*treatment		0.3058
AR(1)	block*treatment	-0.3769
Residual		1.4933

Fit Statistics	
-2 Res Log Likelihood	156.0
AIC (smaller is better)	164.0
AICC (smaller is better)	165.1
BIC (smaller is better)	160.4

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
treatment	3	6	2.72	0.1376
process	4	32	1108.51	<.0001
process*treatment	12	32	0.92	0.5404

Least Squares Means						
Effect	**process**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
process	DF1	26.2917	0.4022	32	65.37	<.0001
process	DF2	29.9250	0.4022	32	74.41	<.0001
process	DF3	30.4500	0.4022	32	75.71	<.0001
process	SM	5.9000	0.4022	32	14.67	<.0001
process	UF	19.6917	0.4022	32	48.96	<.0001

Differences of Least Squares Means									
Effect	**process**	**_process**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**	**Adj P**
process	DF1	DF2	-3.6333	0.5854	32	-6.21	<.0001	Tukey-Kramer	<.0001
process	DF1	DF3	-4.1583	0.4621	32	-9.00	<.0001	Tukey-Kramer	<.0001
process	DF1	SM	20.3917	0.5121	32	39.82	<.0001	Tukey-Kramer	<.0001
process	DF1	UF	6.6000	0.4938	32	13.37	<.0001	Tukey-Kramer	<.0001

Differences of Least Squares Means									
Effect	**process**	**_process**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**	**Adj P**
process	DF2	DF3	-0.5250	0.5854	32	-0.90	0.3765	Tukey-Kramer	0.8960
process	DF2	SM	24.0250	0.4621	32	51.99	<.0001	Tukey-Kramer	<.0001
process	DF2	UF	10.2333	0.5121	32	19.98	<.0001	Tukey-Kramer	<.0001
process	DF3	SM	24.5500	0.5854	32	41.94	<.0001	Tukey-Kramer	<.0001
process	DF3	UF	10.7583	0.4621	32	23.28	<.0001	Tukey-Kramer	<.0001
process	SM	UF	-13.7917	0.5854	32	-23.56	<.0001	Tukey-Kramer	<.0001

Appendix C: Data and Minitab Output for Solubility (1h)

Table 10: Solubility after one hour (1h) reconstitution on laboratory stage mixer. Means and standard deviations were calculated from two measurements.

Block	Treatment	Replicate	Solubility (%)	pH	Temperature (°C)
1	1	1	58.72 ± 0.47	7.49	23.5
1	2	1	68.45 ± 0.87	7.69	24.6
1	3	1	92.03 ± 0.46	7.73	23.5
1	4	1	97.50 ± 0.70	7.74	22.7
2	1	1	57.65 ± 1.03	7.48	22.8
2	2	1	69.82 ± 0.40	7.70	24.3
2	3	1	92.99 ± 0.11	7.73	22.4
2	4	1	95.98 ± 1.14	7.73	23.4
3	1	1	62.45 ± 1.53	7.51	21.7
3	2	1	66.31 ± 1.73	7.70	23.4
3	3	1	93.56 ± 1.09	7.72	22.9
3	4	1	95.42± 0.69	7.72	21.8
1	1	2	56.22 ± 0.81	7.51	23.0
1	2	2	66.68 ± 0.98	7.71	23.7
1	3	2	92.45 ± 0.52	7.73	24.6
1	4	2	96.94 ± 0.76	7.75	22.1
2	1	2	59.21 ± 0.93	7.50	23.0
2	2	2	67.65 ± 0.51	7.69	23.8
2	3	2	92.91 ± 0.49	7.72	22.6
2	4	2	95.87 ± 0.68	7.73	22.0
3	1	2	64.59 ± 0.75	7.50	22.3
3	2	2	69.41 ± 1.12	7.70	24.0
3	3	2	91.23 ± 0.68	7.72	23.3
3	4	2	97.43± 0.91	7.73	22.9

Minitab Output Solubility (1h)

General Linear Model: solubility_1hr versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for solubility_1hr, using Adjusted SS for Tests

Source           DF   Seq SS   Adj SS   Adj MS       F      P
block             2     8.71     8.71     4.35    0.70  0.533
treatment         3  5868.53  5868.53  1956.18  314.45  0.000
block*treatment   6    37.33    37.33     6.22    3.67  0.026
```

```
Error            12    20.35    20.35     1.70
Total            23  5934.91

S = 1.30212   R-Sq = 99.66%   R-Sq(adj) = 99.34%
```

Minitab Output for no Interaction between block and treatment

General Linear Model: solubility_1hr versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for solubility_1hr, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS       F      P
block       2     8.7     8.7     4.4    1.36  0.282
treatment   3  5868.5  5868.5  1956.2  610.54  0.000
Error      18    57.7    57.7     3.2
Total      23  5934.9


S = 1.78998   R-Sq = 99.03%   R-Sq(adj) = 98.76%


Unusual Observations for solubility_1hr

Obs  solubility_1hr      Fit  SE Fit  Residual  St Resid
 21         64.5900  60.6287  0.8950    3.9613      2.56 R

R denotes an observation with a large standardized residual.


Least Squares Means for solubility_1hr

treatment   Mean
1          59.81
2          68.05
3          92.53
4          96.52


Grouping Information Using Tukey Method and 99.0% Confidence

treatment  N  Mean  Grouping
4          6  96.5  A
3          6  92.5    B
2          6  68.1      C
1          6  59.8        D

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable solubility_1hr
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

treatment   Lower  Center  Upper  +---------+---------+---------+------
2           4.527   8.247  11.97      (--*--)
```

```
3          29.002  32.722  36.44                          (--*--)
4          32.997  36.717  40.44                             (---*--)
                                 +---------+---------+---------+------
                                 0         12        24        36


treatment = 2  subtracted from:

treatment  Lower  Center  Upper  +---------+---------+---------+------
3          20.76   24.48  28.19                  (--*--)
4          24.75   28.47  32.19                      (--*--)
                                 +---------+---------+---------+------
                                 0         12        24        36


treatment = 3  subtracted from:

treatment   Lower  Center  Upper  +---------+---------+---------+------
4          0.2755   3.995  7.715  (--*--)
                                  +---------+---------+---------+------
                                  0         12        24        36


Tukey Simultaneous Tests
Response Variable solubility_1hr
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
2               8.247       1.033    7.980    0.0000
3              32.722       1.033   31.663    0.0000
4              36.717       1.033   35.528    0.0000


treatment = 2  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
3              24.48       1.033    23.68    0.0000
4              28.47       1.033    27.55    0.0000


treatment = 3  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
4              3.995       1.033    3.866    0.0057
```

Regression Analysis: solubility_1hr versus pH_1hr

```
The regression equation is
solubility_1hr = - 915 + 130 pH_1hr


Predictor    Coef  SE Coef      T      P
Constant   -914.7    160.2  -5.71  0.000
pH_1hr     129.69    20.90   6.21  0.000


S = 9.90324   R-Sq = 63.6%   R-Sq(adj) = 62.0%
```

```
Analysis of Variance

Source          DF      SS      MS      F      P
Regression       1  3777.3  3777.3  38.51  0.000
Residual Error  22  2157.6    98.1
Total           23  5934.9
```

General Linear Model: pH_1hr versus treatment

```
Factor     Type   Levels  Values
treatment  fixed       4  1, 2, 3, 4


Analysis of Variance for pH_1hr, using Adjusted SS for Tests

Source     DF    Seq SS    Adj SS    Adj MS       F      P
treatment   3  0.222913  0.222913  0.074304  900.66  0.000
Error      20  0.001650  0.001650  0.000082
Total      23  0.224563


S = 0.00908295   R-Sq = 99.27%   R-Sq(adj) = 99.16%


Unusual Observations for pH_1hr

Obs   pH_1hr      Fit   SE Fit  Residual  St Resid
  5  7.48000  7.49833  0.00371  -0.01833     -2.21 R
 16  7.75000  7.73333  0.00371   0.01667      2.01 R

R denotes an observation with a large standardized residual.


Least Squares Means for pH_1hr

treatment   Mean   SE Mean
1          7.498  0.003708
2          7.698  0.003708
3          7.725  0.003708
4          7.733  0.003708


Grouping Information Using Tukey Method and 99.0% Confidence

treatment  N  Mean  Grouping
4          6   7.7  A
3          6   7.7  A
2          6   7.7    B
1          6   7.5      C

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable pH_1hr
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

treatment   Lower  Center   Upper   -+---------+---------+---------+-----
2          0.1814  0.2000  0.2186                          (-*-)
3          0.2081  0.2267  0.2453                             (-*--)
```

```
4          0.2164  0.2350  0.2536                                (-*--)
                                   -+---------+---------+---------+-----
                                  0.000     0.080     0.160     0.240
```

treatment = 2 subtracted from:

```
treatment     Lower   Center    Upper   -+---------+---------+---------+-----
3          0.008052  0.02667  0.04528     (-*--)
4          0.016385  0.03500  0.05361       (-*--)
                                        -+---------+---------+---------+-----
                                       0.000     0.080     0.160     0.240
```

treatment = 3 subtracted from:

```
treatment     Lower    Center    Upper   -+---------+---------+---------+-----
4          -0.01028  0.008333  0.02695   (-*-)
                                         -+---------+---------+---------+-----
                                        0.000     0.080     0.160     0.240
```

Tukey Simultaneous Tests
Response Variable pH_1hr
All Pairwise Comparisons among Levels of treatment
treatment = 1 subtracted from:

treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	0.2000	0.005244	38.14	0.0000
3	0.2267	0.005244	43.22	0.0000
4	0.2350	0.005244	44.81	0.0000

treatment = 2 subtracted from:

treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	0.02667	0.005244	5.085	0.0003
4	0.03500	0.005244	6.674	0.0000

treatment = 3 subtracted from:

treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	0.008333	0.005244	1.589	0.4069**Z**

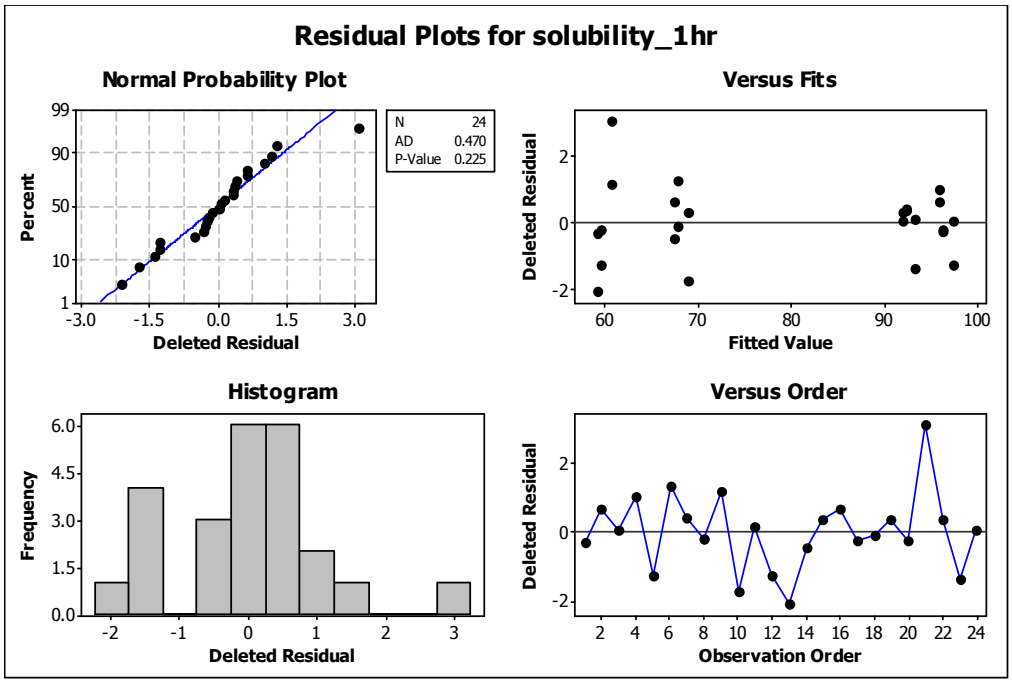


Figure 57: Residual plots for solubility at 1h; Top Left: Normal probability plot for solubility at 1 h (p = 0.225); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

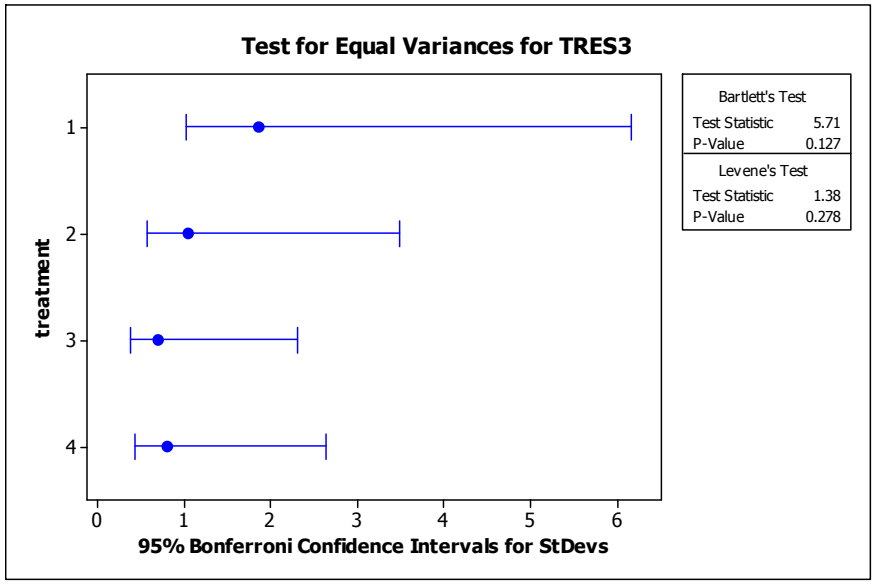


Figure 58: Plot of Variance for Solubility 1h reconstitution; Bartlett's Test statistic = 5.71, p-value = 0.127; Levene's Test statistic = 1.38, p-value = 0.278

General Regression Analysis: solubility_1hr versus conc-75, block

Regression Equation

block
1 solubility_1hr = 78.6238 + 0.26925 conc-75

2 solubility_1hr = 79.01 + 0.26925 conc-75

3 solubility_1hr = 80.05 + 0.26925 conc-75

Coefficients

Term	Coef	SE Coef	T	P	95% CI
Constant	79.2279	1.00937	78.4924	0.000	(77.1224, 81.3334)
conc-75	0.2693	0.01806	14.9118	0.000	(0.2316, 0.3069)
block					
1	-0.6042	1.42747	-0.4232	0.677	(-3.5818, 2.3735)
2	-0.2179	1.42747	-0.1527	0.880	(-3.1956, 2.7597)

Summary of Model

S = 4.94489 R-Sq = 91.76% R-Sq(adj) = 90.52%
PRESS = 672.714 R-Sq(pred) = 88.67%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	5445.87	5445.87	1815.29	74.239	0.000000
conc-75	1	5437.17	5437.17	5437.17	222.362	0.000000
block	2	8.71	8.71	4.35	0.178	0.838225
Error	20	489.04	489.04	24.45		
Lack-of-Fit	8	468.69	468.69	58.59	34.554	0.000000
Pure Error	12	20.35	20.35	1.70		
Total	23	5934.91				

Fits and Diagnostics for Unusual Observations

No unusual observations

Appendix D: Data and Minitab Output for Solubility (3h)

Table 11: Solubility after three hours (3h) reconstitution on laboratory stage mixer. Means and standard deviations were calculated from two replicates

Block	Treatment	Replicate	Solubility (%)	pH	Temperature (°C)
1	1	1	67.00 ± 0.56	7.35	23.0
1	2	1	95.34 ± 0.97	7.57	23.8
1	3	1	99.45 ± 0.34	7.62	22.5
1	4	1	99.43 ± 0.65	7.67	24.1
2	1	1	62.92 ± 1.45	7.32	23.2
2	2	1	96.75 ± 0.76	7.55	22.3
2	3	1	97.85 ± 0.43	7.62	21.4
2	4	1	97.21 ± 0.60	7.69	23.0
3	1	1	63.11 ± 1.91	7.29	21.5
3	2	1	92.44 ± 0.56	7.52	22.6
3	3	1	96.75 ± 0.85	7.59	23.4
3	4	1	99.48 ± 0.34	7.65	23.4
1	1	2	63.68 ± 1.43	7.33	21.5
1	2	2	94.20 ± 0.60	7.56	24.0
1	3	2	97.18 ± 0.52	7.63	22.0
1	4	2	98.01 ± 0.31	7.66	23.2
2	1	2	60.18 ± 1.21	7.30	21.1
2	2	2	94.91 ± 0.51	7.57	24.3
2	3	2	99.56 ± 0.12	7.64	23.5
2	4	2	98.24 ± 0.42	7.67	24.5
3	1	2	67.72 ± 1.58	7.29	23.4
3	2	2	92.98 ± 0.56	7.56	23.2
3	3	2	97.04 ± 0.45	7.58	21.5
3	4	2	98.24 ± 0.51	7.63	22.9

Minitab Output for block*treatment Interaction

General Linear Model: solubility_3hr versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for solubility_3hr, using Adjusted SS for Tests

Source           DF   Seq SS   Adj SS   Adj MS       F      P
block             2     3.63     3.63     1.82    0.35  0.718
treatment         3  4912.30  4912.30  1637.43  315.53  0.000
block*treatment   6    31.14    31.14     5.19    2.16  0.120
Error            12    28.77    28.77     2.40
Total            23  4975.83
```

```
S = 1.54832   R-Sq = 99.42%   R-Sq(adj) = 98.89%


Unusual Observations for solubility_3hr

Obs  solubility_3hr      Fit  SE Fit  Residual  St Resid
  9         63.1100  65.4150  1.0948   -2.3050     -2.11 R
 21         67.7200  65.4150  1.0948    2.3050      2.11 R

R denotes an observation with a large standardized residual.
```

Minitab Output for no Interaction between block and treatment

General Linear Model: solubility_3h versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for solubility_3h, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS   Adj MS       F      P
block       2     3.63     3.63     1.82    0.55  0.589
treatment   3  4912.30  4912.30  1637.43  492.02  0.000
Error      18    59.90    59.90     3.33
Total      23  4975.83


S = 1.82428   R-Sq = 98.80%   R-Sq(adj) = 98.46%


Unusual Observations for solubility_3h

Obs  solubility_3h      Fit  SE Fit  Residual  St Resid
 17        60.1800  63.8179  0.9121   -3.6379     -2.30 R
 21        67.7200  63.8354  0.9121    3.8846      2.46 R

R denotes an observation with a large standardized residual.


Least Squares Means for solubility_3h

treatment   Mean
1          64.10
2          94.44
3          97.97
4          98.44


Grouping Information Using Tukey Method and 99.0% Confidence

treatment  N  Mean  Grouping
4          6  98.4  A
3          6  98.0  A B
2          6  94.4    B
1          6  64.1      C

Means that do not share a letter are significantly different.
```

```
Tukey 99.0% Simultaneous Confidence Intervals
Response Variable solubility_3h
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

treatment  Lower  Center  Upper  ---+---------+---------+---------+---
2          26.54   30.34  34.13                        (--*--)
3          30.08   33.87  37.66                           (--*--)
4          30.54   34.33  38.12                           (---*--)
                                ---+---------+---------+---------+---
                                   0        12        24        36


treatment = 2  subtracted from:

treatment    Lower  Center  Upper  ---+---------+---------+---------+---
3          -0.2558   3.535  7.326     (--*--)
4           0.2075   3.998  7.789     (--*--)
                                  ---+---------+---------+---------+---
                                     0        12        24        36


treatment = 3  subtracted from:

treatment   Lower  Center  Upper  ---+---------+---------+---------+---
4          -3.327  0.4633  4.254  (--*---)
                                  ---+---------+---------+---------+---
                                     0        12        24        36


Tukey Simultaneous Tests
Response Variable solubility_3h
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
2               30.34       1.053    28.80    0.0000
3               33.87       1.053    32.16    0.0000
4               34.33       1.053    32.60    0.0000


treatment = 2  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
3               3.535       1.053    3.356    0.0169
4               3.998       1.053    3.796    0.0066


treatment = 3  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
4              0.4633       1.053   0.4399    0.9707
```

Regression Analysis: solubility_3h versus pH_3h

```
The regression equation is
solubility_3h = - 689 + 103 pH_3h
```

```
Predictor     Coef  SE Coef       T      P
Constant   -688.81    41.53  -16.58  0.000
pH_3h      103.180    5.511   18.72  0.000


S = 3.65446   R-Sq = 94.1%   R-Sq(adj) = 93.8%

Analysis of Variance

Source          DF      SS      MS       F      P
Regression       1  4682.0  4682.0  350.58  0.000
Residual Error  22   293.8    13.4
Total           23  4975.8

Unusual Observations

Obs  pH_3h  solubility_3h      Fit  SE Fit  Residual  St Resid
  8   7.69         97.210  104.643   1.131    -7.433     -2.14R

R denotes an observation with a large standardized residual.
```

General Linear Model: pH_3h versus treatment

```
Factor     Type   Levels  Values
treatment  fixed       4  1, 2, 3, 4


Analysis of Variance for pH_3h, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS   Adj MS       F      P
treatment   3  0.43028  0.43028  0.14343  301.95  0.000
Error      20  0.00950  0.00950  0.00048
Total      23  0.43978


S = 0.0217945   R-Sq = 97.84%   R-Sq(adj) = 97.52%


Least Squares Means for pH_3h

treatment   Mean   SE Mean
1          7.313  0.008898
2          7.555  0.008898
3          7.613  0.008898
4          7.662  0.008898


Grouping Information Using Tukey Method and 99.0% Confidence

treatment  N  Mean  Grouping
4          6   7.7  A
3          6   7.6    B
2          6   7.6      C
1          6   7.3        D

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable pH_3h
All Pairwise Comparisons among Levels of treatment
```

```
treatment = 1  subtracted from:

treatment   Lower  Center   Upper    +---------+---------+---------+------
2          0.1970  0.2417  0.2863                  (---*---)
3          0.2553  0.3000  0.3447                       (---*---)
4          0.3037  0.3483  0.3930                           (---*---)
                                    +---------+---------+---------+------
                                  0.00      0.12      0.24      0.36


treatment = 2  subtracted from:

treatment    Lower   Center   Upper    +---------+---------+---------+------
3          0.01367  0.05833  0.1030     (---*---)
4          0.06200  0.10667  0.1513         (---*---)
                                      +---------+---------+---------+------
                                    0.00      0.12      0.24      0.36


treatment = 3  subtracted from:

treatment     Lower   Center    Upper    +---------+---------+---------+------
4          0.003668  0.04833  0.09300    (---*---)
                                        +---------+---------+---------+------
                                      0.00      0.12      0.24      0.36


Tukey Simultaneous Tests
Response Variable pH_3h
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
2              0.2417     0.01258    19.21    0.0000
3              0.3000     0.01258    23.84    0.0000
4              0.3483     0.01258    27.68    0.0000


treatment = 2  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
3             0.05833     0.01258    4.636    0.0009
4             0.10667     0.01258    8.477    0.0000


treatment = 3  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
   4              0.04833     0.01258    3.841    0.0052
```

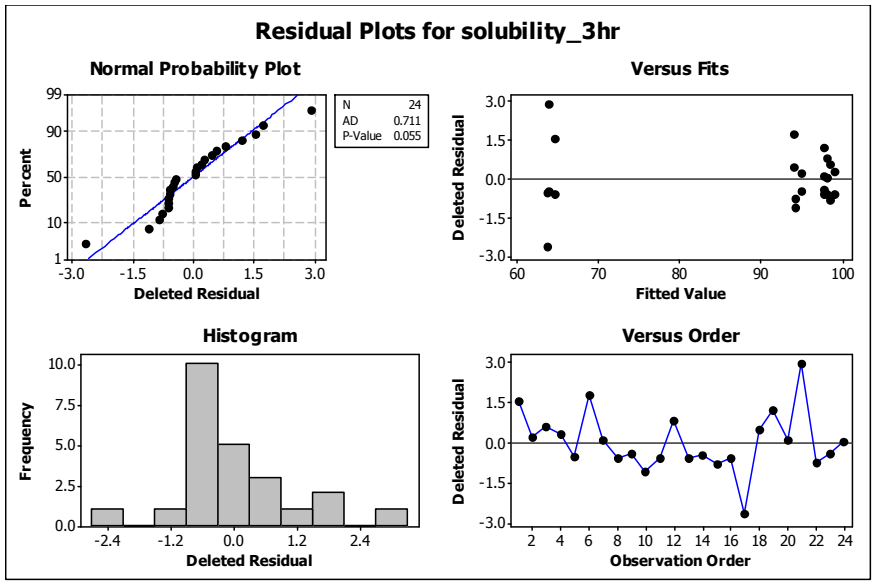


Figure 59: Residual plots for solubility at 3h; Top Left: Normal probability plot for solubility at 3 h (p = 0.055); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

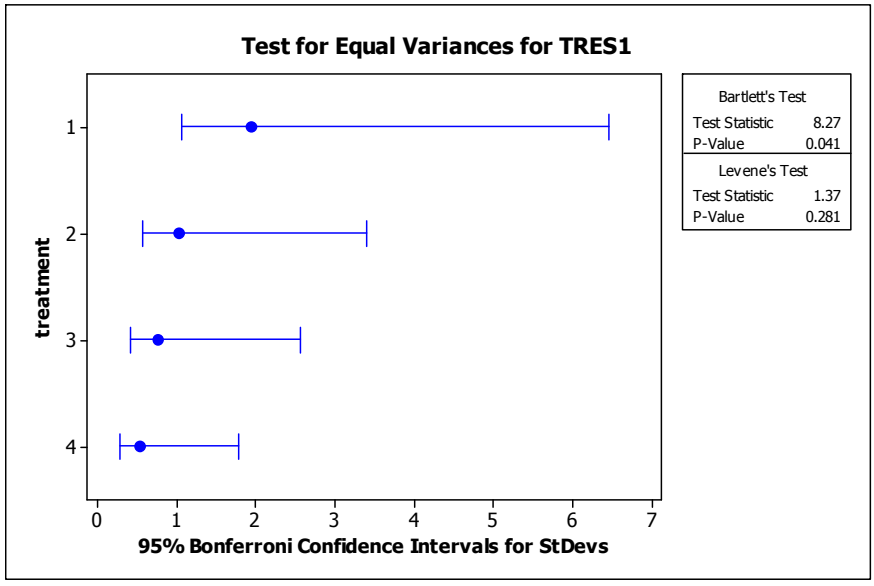


Figure 60: Plot of Variance for Solubility 3h reconstitution; Bartlett's Test statistic = 8.27, p-value = 0.041; Levene's Test statistic = 1.37, p-value = 0.281

General Regression Analysis: solubility_3hr versus log2(conc+1), block

Regression Equation

block
1 solubility_3hr = 65.0903 + 4.94575 log2(conc+1)

2 solubility_3hr = 64.2566 + 4.94575 log2(conc+1)

3 solubility_3hr = 64.2741 + 4.94575 log2(conc+1)

Coefficients

Term	Coef	SE Coef	T	P	99% CI	VIF
Constant	64.5403	0.920702	70.0991	0.000	(61.9206, 67.1601)	
log2(conc+1)	4.9458	0.162188	30.4940	0.000	(4.4843, 5.4072)	1.00000
block						
1	0.5500	0.660463	0.8327	0.415	(-1.3292, 2.4292)	1.33333
2	-0.2837	0.660463	-0.4296	0.672	(-2.1630, 1.5955)	1.33333

Summary of Model

S = 2.28791 R-Sq = 97.90% R-Sq(adj) = 97.58%
PRESS = 156.441 R-Sq(pred) = 96.86%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	4871.14	4871.14	1623.71	310.192	0.000000
log2(conc+1)	1	4867.51	4867.51	4867.51	929.883	0.000000
block	2	3.63	3.63	1.82	0.347	0.711078
Error	20	104.69	104.69	5.23		
Lack-of-Fit	8	75.92	75.92	9.49	3.959	0.016253
Pure Error	12	28.77	28.77	2.40		
Total	23	4975.83				

Fits and Diagnostics for Unusual Observations

Obs	solubility_3hr	Fit	SE Fit	Residual	St Resid	
6	96.75	92.3110	0.81874	4.43900	2.07779	R
17	60.18	64.2566	1.13309	-4.07660	-2.05099	R

R denotes an observation with a large standardized residual.

Appendix E: Data and Minitab Output for Insolubility Index

Table 12: Solubility as determined by ISI method. Means and standard deviations were calculated from two measurements.

Block	Treatment	ISI (ml sediment)
1	1	9.25 ± 0.35
1	2	6.25 ± 0.35
1	3	2.20 ± 0.28
1	4	0.50 ± 0.00
2	1	9.70 ± 0.21
2	2	7.50 ± 0.71
2	3	3.30± 0.42
2	4	0.50 ± 0.00
3	1	11.25 ± 0.35
3	2	7.40 ± 0.14
3	3	2.25 ± 0.35
3	4	0.75 ± 0.35

Minitab output for Insolubility Index

General Linear Model: ISI (ml) versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for ISI (ml), using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS  Adj MS       F      P
block       2    1.680    1.680   0.840    2.19  0.193
treatment   3  165.602  165.602  55.201  144.03  0.000
Error       6    2.300    2.300   0.383
Total      11  169.582


S = 0.619083   R-Sq = 98.64%   R-Sq(adj) = 97.51%


Least Squares Means for ISI (ml)

treatment     Mean
1          10.0667
2           7.0500
3           2.5833
4           0.5833


Grouping Information Using Tukey Method and 99.0% Confidence

treatment  N  Mean  Grouping
1          3  10.1  A
```

```
2          3   7.0    B
3          3   2.6      C
4          3   0.6      C

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable ISI (ml)
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

treatment   Lower  Center   Upper  ----+---------+---------+---------+--
2          -5.53  -3.017  -0.504                  (------*-------)
3         -10.00  -7.483  -4.971       (-------*------)
4         -12.00  -9.483  -6.971  (------*------)
                                  ----+---------+---------+---------+--
                                  -10.5      -7.0      -3.5       0.0


treatment = 2  subtracted from:

treatment   Lower  Center   Upper  ----+---------+---------+---------+--
3         -6.979  -4.467  -1.954               (------*------)
4         -8.979  -6.467  -3.954         (-------*------)
                                  ----+---------+---------+---------+--
                                  -10.5      -7.0      -3.5       0.0


treatment = 3  subtracted from:

treatment   Lower  Center   Upper  ----+---------+---------+---------+--
4         -4.513  -2.000  0.5127                       (------*------)
                                  ----+---------+---------+---------+--
                                  -10.5      -7.0      -3.5       0.0


Tukey Simultaneous Tests
Response Variable ISI (ml)
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
2              -3.017      0.5055    -5.97    0.0040
3              -7.483      0.5055   -14.80    0.0000
4              -9.483      0.5055   -18.76    0.0000


treatment = 2  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
3              -4.467      0.5055    -8.84    0.0005
4              -6.467      0.5055   -12.79    0.0001


treatment = 3  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
4              -2.000      0.5055   -3.957    0.0286
```

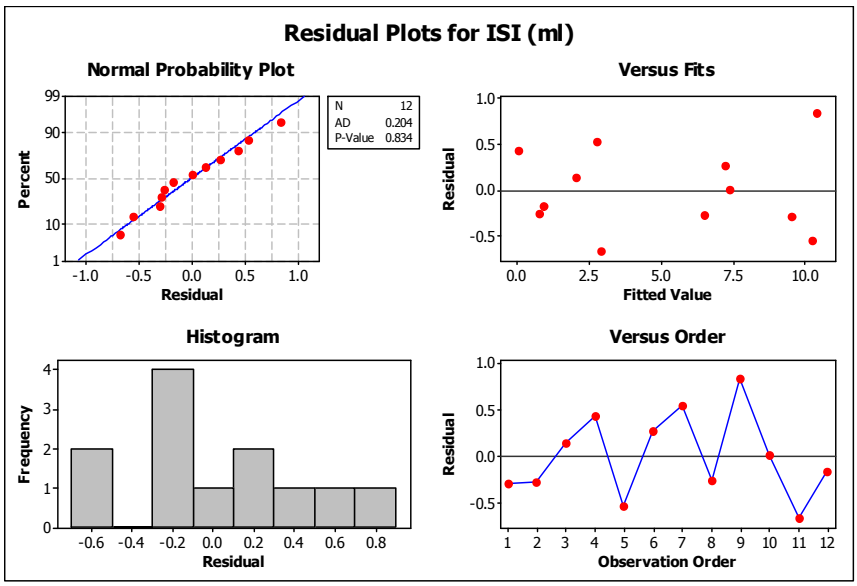


Figure 61: Residual plots for ISI; Top Left: Normal probability plot for ISI (p = 0.834); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

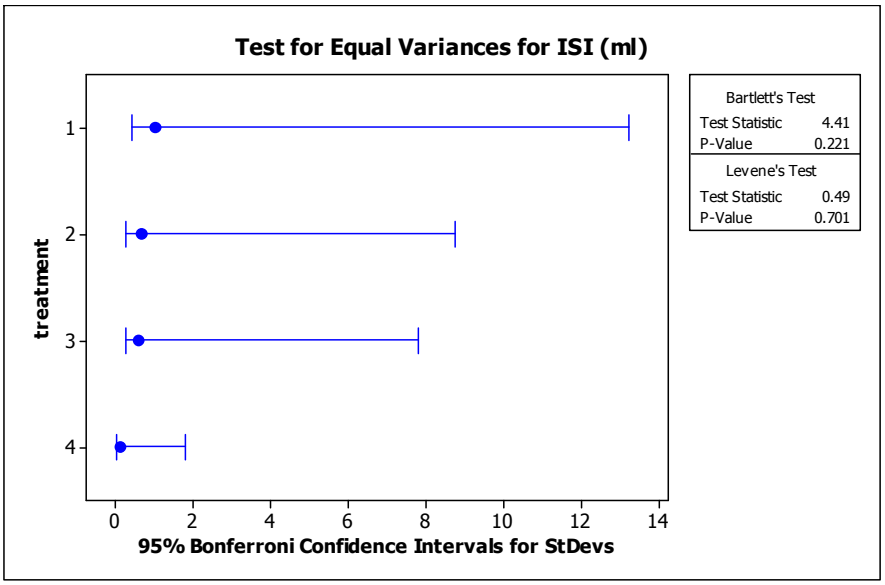


Figure 62: Plot of Variance for ISI; Bartlett's Test statistic = 4.41, p-value = 0.221; Levene's Test statistic = 0.49, p-value = 0.701

General Regression Analysis: ISI (ml) versus concentration, block

Regression Equation

block
1 ISI (ml) = 9.4875 - 0.0658333 concentration

2 ISI (ml) = 10.1875 - 0.0658333 concentration

3 ISI (ml) = 10.35 - 0.0658333 concentration

Coefficients

Term	Coef	SE Coef	T	P	95% CI	VIF
Constant	10.0083	0.395974	25.2753	0.000	(9.09522, 10.9215)	
concentration	-0.0658	0.004233	-15.5519	0.000	(-0.07559, -0.0561)	1.00000
block						
1	-0.5208	0.334659	-1.5563	0.158	(-1.29256, 0.2509)	1.33333
2	0.1792	0.334659	0.5354	0.607	(-0.59256, 0.9509)	1.33333

Summary of Model

S = 0.819743 R-Sq = 96.83% R-Sq(adj) = 95.64%
PRESS = 11.8352 R-Sq(pred) = 93.02%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	164.206	164.206	54.735	81.454	0.000002
concentration	1	162.526	162.526	162.526	241.862	0.000000
block	2	1.680	1.680	0.840	1.250	0.336889
Error	8	5.376	5.376	0.672		
Total	11	169.582				

Fits and Diagnostics for Unusual Observations

Obs	ISI (ml)	Fit	SE Fit	Residual	St Resid	
11	2.25	3.76667	0.423314	-1.51667	-2.16054	R

R denotes an observation with a large standardized residual.

Appendix F: Data and Minitab Output for Anema et al. (2006)

Table 13: Solubility as determined by method of Anema et al. (2006). Means and standard deviations were calculated from two measurements.

Block	Treatment	Solubility (%)
1	1	48.16 ± 1.47
1	2	59.47 ± 0.77
1	3	83.71 ± 1.81
1	4	97.40 ± 0.52
2	1	48.02 ± 1.10
2	2	63.77 ± 1.85
2	3	81.61 ± 1.06
2	4	93.56 ± 0.24
3	1	42.22 ± 2.19
3	2	54.02 ± 1.83
3	3	91.84 ± 1.04
3	4	97.64 ± 0.73

Minitab output for Solubility using method of Anema et al. (2006)

General Linear Model: solubility_an versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for solubility_an, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS   Adj MS      F      P
block       2     1.15     1.15     0.58   0.02  0.975
treatment   3  4828.60  4828.60  1609.53  69.75  0.000
Error       6   138.45   138.45    23.08
Total      11  4968.20


S = 4.80365   R-Sq = 97.21%   R-Sq(adj) = 94.89%


Least Squares Means for solubility_an

treatment   Mean
1          46.13
2          59.09
3          85.72
4          96.20


Grouping Information Using Tukey Method and 99.0% Confidence

treatment  N  Mean  Grouping
4          3  96.2  A
```

```
3          3  85.7  A
2          3  59.1    B
1          3  46.1    B

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable solubility_an
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

treatment   Lower  Center  Upper  ----+---------+---------+---------+--
2          -6.544   12.95  32.45   (-------*-------)
3          20.090   39.59  59.08              (-------*-------)
4          30.570   50.07  69.56                  (-------*-------)
                                  ----+---------+---------+---------+--
                                      0        25        50        75


treatment = 2  subtracted from:

treatment   Lower  Center  Upper  ----+---------+---------+---------+--
3           7.136   26.63  46.13         (-------*------)
4          17.616   37.11  56.61             (-------*-------)
                                  ----+---------+---------+---------+--
                                      0        25        50        75


treatment = 3  subtracted from:

treatment   Lower  Center  Upper  ----+---------+---------+---------+--
4          -9.017   10.48  29.98  (-------*-------)
                                  ----+---------+---------+---------+--
                                      0        25        50        75


Tukey Simultaneous Tests
Response Variable solubility_an
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
2               12.95       3.922    3.303    0.0602
3               39.59       3.922   10.093    0.0002
4               50.07       3.922   12.765    0.0001


treatment = 2  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
3               26.63       3.922    6.790    0.0020
4               37.11       3.922    9.462    0.0003


treatment = 3  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
4               10.48       3.922    2.672    0.1277
```

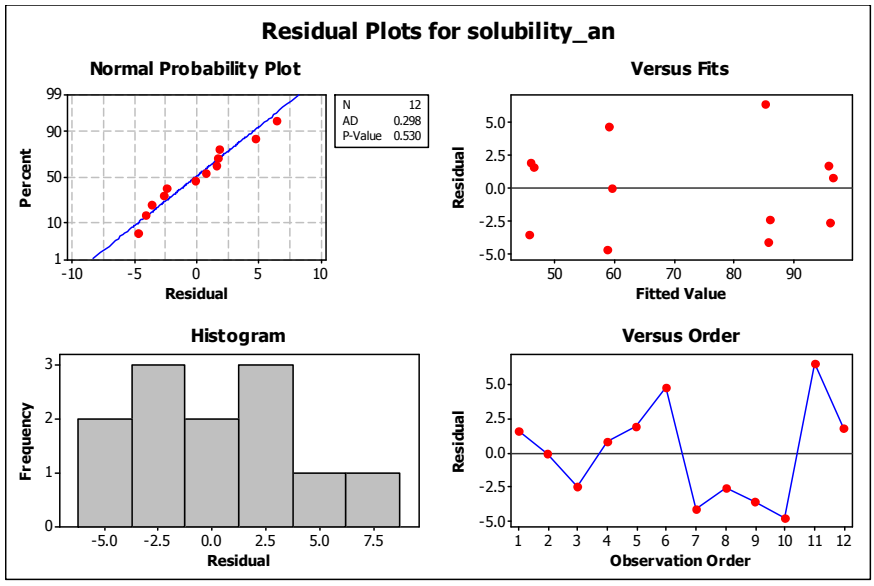


Figure 63: Residual plots for Solubility according to method of Anema et al. (2006); Top Left: Normal probability plot (p = 0.530); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

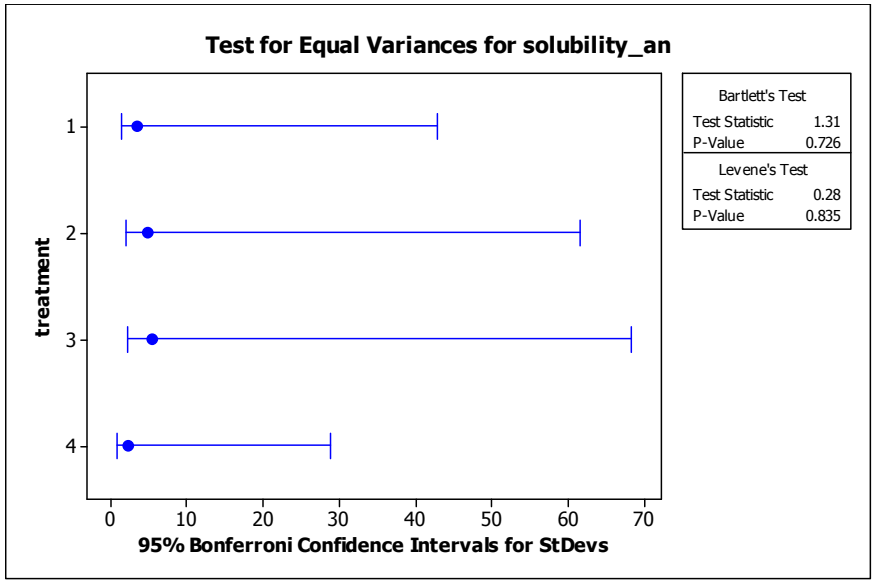


Figure 64: Plot of Variance for Solubility according to method of Anema et al. (2006); Bartlett's Test statistic = 1.31, p-value = 0.726; Levene's Test statistic = 0.28, p-value = 0.835

General Regression Analysis: solubility versus concentration, block

Regression Equation

block
1 solubility = 45.66 + 0.353667 concentration

2 solubility = 45.215 + 0.353667 concentration

3 solubility = 44.905 + 0.353667 concentration

Coefficients

Term	Coef	SE Coef	T	P	95% CI	VIF
Constant	45.2600	2.84004	15.9364	0.000	(38.7109, 51.8091)	
concentration	0.3537	0.03036	11.6486	0.000	(0.2837, 0.4237)	1.00000
block						
1	0.4000	2.40027	0.1666	0.872	(-5.1350, 5.9350)	1.33333
2	-0.0450	2.40027	-0.0187	0.986	(-5.5800, 5.4900)	1.33333

Summary of Model

S = 5.87944 R-Sq = 94.43% R-Sq(adj) = 92.35%
PRESS = 555.811 R-Sq(pred) = 88.81%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	4691.66	4691.66	1563.89	45.241	0.000023
concentration	1	4690.50	4690.50	4690.50	135.690	0.000003
block	2	1.15	1.15	0.58	0.017	0.983506
Error	8	276.54	276.54	34.57		
Total	11	4968.20				

Fits and Diagnostics for Unusual Observations

Obs	solubility	Fit	SE Fit	Residual	St Resid	
11	91.84	80.2717	3.03613	11.5683	2.29765	R

R denotes an observation with a large standardized residual.

Appendix G: Data and Minitab Output for Solubility After Adjustment to Treatment Level 4 pH

Table 14: Solubility after three hours (3h) reconstitution on laboratory stage mixer, when adjusted to treatment 4 pH after 1h mixing. Means and standard deviations were calculated from two replicates.

Block	Treatment	Solubility (%)	pH (initial)	pH (final)	Temperature (°C)
1	1	66.98 ± 1.36	7.52 ± 0.01	7.68 ± 0.02	23.1 ± 0.28
1	2	90.39 ± 1.27	7.63 ± 0.01	7.67 ± 0.03	23.0 ± 0.35
1	3	96.72 ± 1.88	7.69 ± 0.01	7.66 ± 0.01	23.3 ± 0.14
1	4	95.98 ± 1.30	7.73 ± 0.01	7.67 ± 0.00	22.9 ± 0.49
2	1	68.30 ± 2.39	7.52 ± 0.01	7.67 ± 0.01	22.8 ± 0.28
2	2	95.88 ± 2.21	7.63 ± 0.01	7.68 ± 0.01	23.0 ± 0.42
2	3	99.57 ± 0.45	7.68 ± 0.01	7.68 ± 0.01	22.3 ± 0.21
2	4	97.81 ± 1.45	7.72 ± 0.01	7.67 ± 0.00	23.0 ± 0.21
3	1	62.09 ± 1.18	7.52 ± 0.01	7.69 ± 0.01	22.4 ± 0.35
3	2	92.51 ± 2.67	7.63 ± 0.01	7.67 ± 0.02	23.2 ± 0.77
3	3	95.46 ± 1.12	7.69 ± 0.01	7.67 ± 0.01	23.1 ± 0.35
3	4	99.36 ± 0.42	7.73 ± 0.01	7.67 ± 0.00	23.5 ± 1.06

Minitab Output for Solubility After Adjustment to Treatment Level 4 pH

General Linear Model: solubility versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for solubility, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS  Adj MS       F      P
block       2    23.32    23.32   11.66    2.50  0.163
treatment   3  2090.47  2090.47  696.82  149.26  0.000
Error       6    28.01    28.01    4.67
Total      11  2141.80


S = 2.16066   R-Sq = 98.69%   R-Sq(adj) = 97.60%


Least Squares Means for solubility

treatment   Mean
1          65.79
2          92.93
3          97.25
4          97.72


Grouping Information Using Tukey Method and 99.0% Confidence
```

```
treatment  N  Mean  Grouping
4          3  97.7  A
3          3  97.2  A
2          3  92.9  A
1          3  65.8    B

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable solubility
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

treatment  Lower  Center  Upper  ------+---------+---------+---------+
2          18.37   27.14  35.91                  (-----*-----)
3          22.69   31.46  40.23                     (-----*-----)
4          23.16   31.93  40.70                     (-----*-----)
                                ------+---------+---------+---------+
                                      0        15        30        45


treatment = 2  subtracted from:

treatment   Lower  Center  Upper  ------+---------+---------+---------+
3          -4.446   4.323  13.09     (-----*-----)
4          -3.980   4.790  13.56     (-----*-----)
                                ------+---------+---------+---------+
                                      0        15        30        45


treatment = 3  subtracted from:

treatment   Lower  Center  Upper  ------+---------+---------+---------+
4          -8.303  0.4667  9.236  (-----*-----)
                                ------+---------+---------+---------+
                                      0        15        30        45


Tukey Simultaneous Tests
Response Variable solubility
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
2               27.14       1.764    15.38    0.0000
3               31.46       1.764    17.83    0.0000
4               31.93       1.764    18.10    0.0000


treatment = 2  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
3               4.323       1.764    2.451    0.1668
4               4.790       1.764    2.715    0.1212


treatment = 3  subtracted from:

           Difference       SE of           Adjusted
```

```
treatment     of Means  Difference  T-Value   P-Value
    4               0.4667       1.764   0.2645    0.9928
    5
```

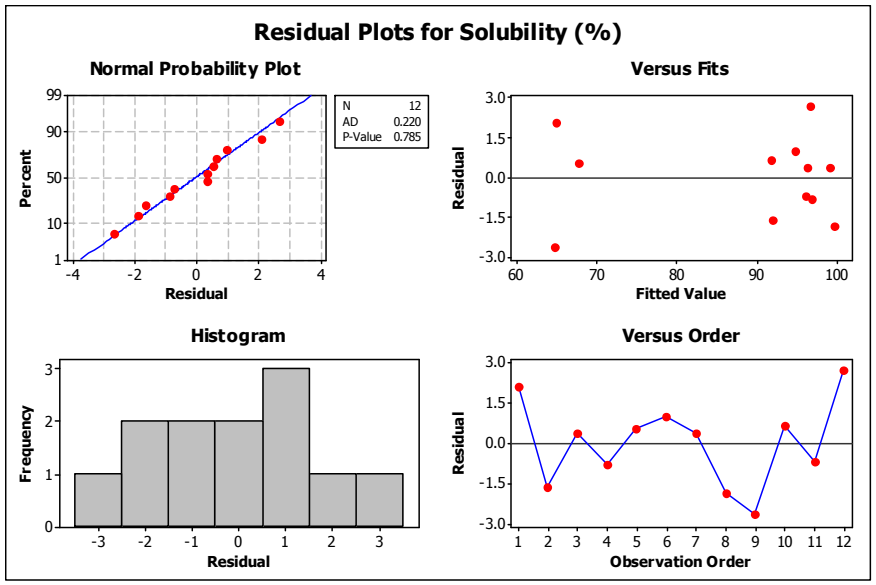


Figure 65: Residual plots for Solubility after adjustment to Treatment level 4 pH; Top Left: Normal probability plot (p = 0.785); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

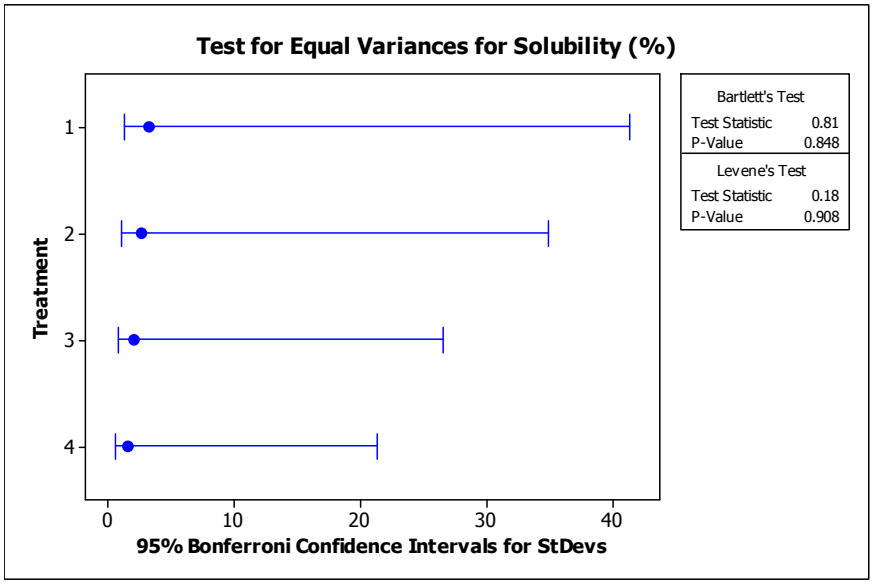


Figure 66: Plot of Variance for Solubility after adjustment to Treatment level 4 pH; Bartlett's Test statistic = 0.81, p-value = 0.848; Levene's Test statistic = 0.18, p-value = 0.908

Appendix H: Data and Minitab Output for Solubility After Adjustment to Control Level 4 pH

Table 15: Solubility after three hours (3h) reconstitution on laboratory stage mixer, when adjusted to Control pH after 1h mixing. Means and standard deviations were calculated from two replicates

Block	Treatment	Solubility (%)	pH (initial)	pH (final)	Temperature (°C)
1	1	62.70 ± 2.96	7.53 ± 0.01	7.41 ± 0.00	24.0 ± 0.35
1	2	80.04 ± 1.35	7.62 ± 0.01	7.41 ± 0.02	24.3 ± 0.21
1	3	94.83 ± 1.52	7.69 ± 0.01	7.42 ± 0.01	23.7 ± 0.42
1	4	97.95 ± 2.01	7.73 ± 0.01	7.40 ± 0.02	24.1 ± 0.21
2	1	60.27 ± 1.04	7.52 ± 0.01	7.41 ± 0.01	23.2 ± 0.77
2	2	85.40 ± 1.21	7.63 ± 0.01	7.42 ± 0.02	23.6 ± 1.06
2	3	96.21 ± 1.28	7.68 ± 0.01	7.41 ± 0.02	22.5 ± 0.84
2	4	99.11 ± 0.31	7.72 ± 0.01	7.41 ± 0.01	22.2 ± 0.92
3	1	66.86 ± 1.18	7.53 ± 0.01	7.41 ± 0.01	23.8 ± 0.70
3	2	85.94 ± 1.13	7.63 ± 0.01	7.42 ± 0.03	23.5 ± 0.35
3	3	99.68 ± 0.49	7.69 ± 0.01	7.40 ± 0.02	24.0 ± 0.49
3	4	99.56 ± 0.22	7.73 ± 0.01	7.43 ± 0.02	24.2 ± 0.07

Minitab Output for Solubility After Adjustment to Control Level pH

General Linear Model: solubility versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for solubility, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS  Adj MS       F      P
block       2    35.41    35.41   17.71    4.84  0.056
treatment   3  2416.70  2416.70  805.57  220.22  0.000
Error       6    21.95    21.95    3.66
Total      11  2474.06


S = 1.91259   R-Sq = 99.11%   R-Sq(adj) = 98.37%


Least Squares Means for solubility

treatment   Mean
1          63.28
2          83.79
3          96.91
4          98.87


Grouping Information Using Tukey Method and 99.0% Confidence
```

```
treatment  N  Mean  Grouping
4          3  98.9  A
3          3  96.9  A
2          3  83.8    B
1          3  63.3      C

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable solubility
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

treatment  Lower  Center  Upper  ----+---------+---------+---------+--
2          12.75   20.52  28.28              (----*----)
3          25.87   33.63  41.39                      (-----*-----)
4          27.83   35.60  43.36                        (----*----)
                                ----+---------+---------+---------+--
                                    0        15        30        45


treatment = 2  subtracted from:

treatment  Lower  Center  Upper  ----+---------+---------+---------+--
3          5.351   13.11  20.88         (----*----)
4          7.317   15.08  22.84          (----*----)
                               ----+---------+---------+---------+--
                                   0        15        30        45


treatment = 3  subtracted from:

treatment   Lower  Center  Upper  ----+---------+---------+---------+--
4          -5.796   1.967  9.729  (----*----)
                                   ----+---------+---------+---------+--
                                       0        15        30        45


Tukey Simultaneous Tests
Response Variable solubility
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
2               20.52       1.562    13.14    0.0001
3               33.63       1.562    21.54    0.0000
4               35.60       1.562    22.79    0.0000


treatment = 2  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
3               13.11       1.562    8.397    0.0007
4               15.08       1.562    9.657    0.0003


treatment = 3  subtracted from:

           Difference       SE of           Adjusted
```

```
treatment     of Means  Difference  T-Value   P-Value
4                1.967       1.562    1.259    0.6167
```

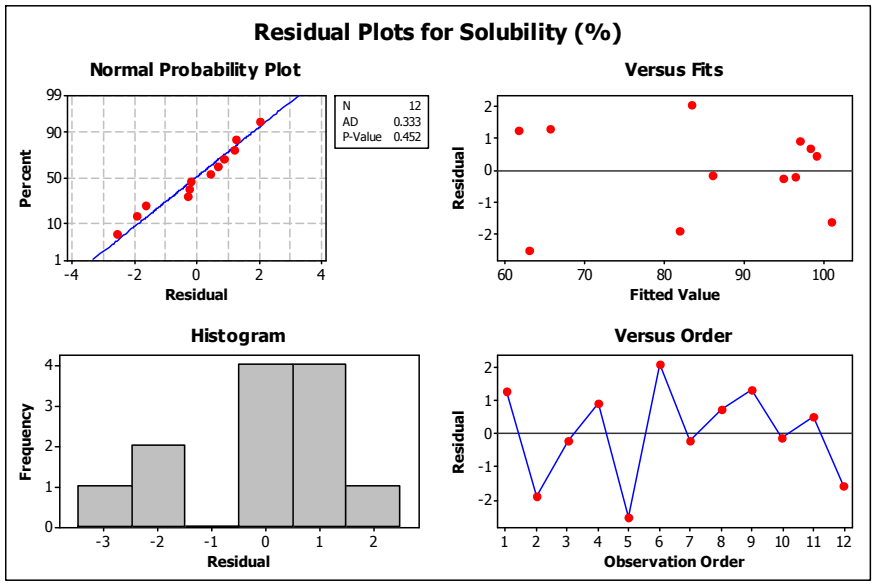


Figure 67: Residual plots for Solubility after adjustment to Control pH; Top Left: Normal probability plot (p = 0.452); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

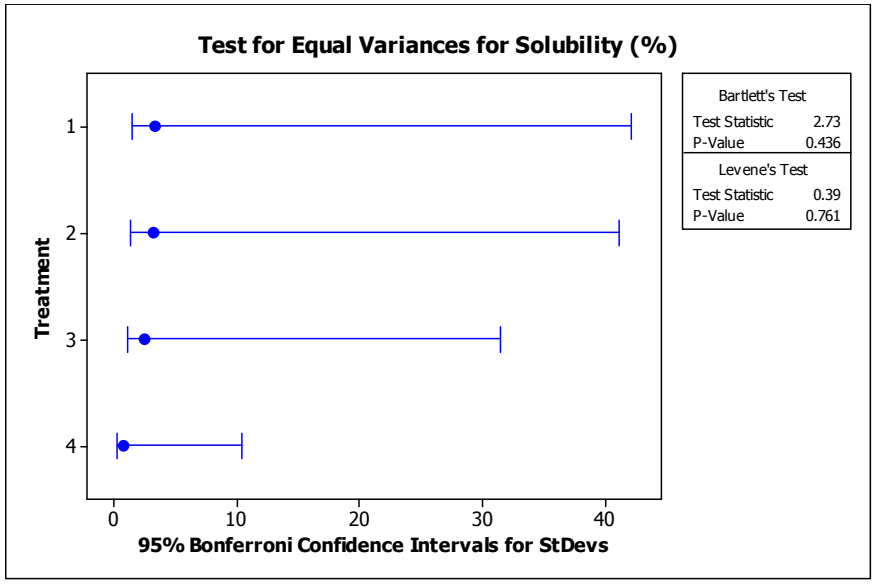


Figure 68: Plot of Variance for Solubility after adjustment to Control pH; Bartlett's Test statistic = 2.73, p-value = 0.436; Levene's Test statistic = 0.39, p-value = 0.761

Appendix I: Proximate Analysis as Determined by FOSS Milkoscan FT2

Table 16: Proximate analysis of products obtained by UF and DF processes during MPC manufacture, determined by FOSS Milkoscan FT2. Means and standard deviations were obtained from duplicate measurements.

Sample	Block	Treatment	Fat	Protein	Casein	Lactose	TS
Milk	1	1	0.22 ± 0.00	3.43 ± 0.01	2.69 ± 0.01	5.10 ± 0.01	9.43 ± 0.01
Milk	1	2	0.21 ± 0.00	3.43 ± 0.00	2.61 ± 0.01	5.09 ± 0.00	9.42 ± 0.01
Milk	1	3	0.22 ± 0.00	3.40 ± 0.00	2.59 ± 0.01	5.06 ± 0.00	9.37 ± 0.00
Milk	1	4	0.21 ± 0.00	3.44 ± 0.01	2.63 ± 0.01	5.09 ± 0.00	9.44 ± 0.00
Milk	2	1	0.17 ± 0.00	3.40 ± 0.01	2.64 ± 0.01	5.06 ± 0.01	9.32 ± 0.01
Milk	2	2	0.21 ± 0.00	3.43 ± 0.00	2.61 ± 0.01	5.09 ± 0.00	9.42 ± 0.03
Milk	2	3	0.23 ± 0.00	3.43 ± 0.00	2.69 ± 0.01	5.12 ± 0.00	9.47 ± 0.01
Milk	2	4	0.17 ± 0.00	3.37 ± 0.01	2.63 ± 0.01	5.06 ± 0.00	9.29 ± 0.01
Milk	3	1	0.17 ± 0.00	3.44 ± 0.01	2.65 ± 0.01	5.12 ± 0.00	9.40 ± 0.00
Milk	3	2	0.17 ± 0.00	3.41 ± 0.00	2.61 ± 0.01	5.09 ± 0.00	9.33 ± 0.01
Milk	3	3	0.18 ± 0.00	3.43 ± 0.01	2.66 ± 0.01	5.14 ± 0.00	9.43 ± 0.01
Milk	3	4	0.17 ± 0.00	3.37 ± 0.00	2.61 ± 0.01	5.05 ± 0.00	9.28 ± 0.01
UF	1	1	0.60 ± 0.01	12.78 ± 0.00	10.23 ± 0.00	4.19 ± 0.00	18.52 ± 0.01
UF	1	2	0.71 ± 0.01	12.53 ± 0.00	10.03 ± 0.01	4.22 ± 0.00	18.47 ± 0.00
UF	1	3	0.78 ± 0.01	13.51 ± 0.01	10.80 ± 0.00	4.15 ± 0.00	19.49 ± 0.04
UF	1	4	0.69 ± 0.00	13.16 ± 0.01	10.48 ± 0.01	4.18 ± 0.00	19.09 ± 0.00
UF	2	1	0.51 ± 0.00	12.45 ± 0.01	9.94 ± 0.01	4.18 ± 0.00	18.06 ± 0.00
UF	2	2	0.50 ± 0.00	12.66 ± 0.01	10.10 ± 0.04	4.18 ± 0.00	18.26 ± 0.03
UF	2	3	0.79 ± 0.00	13.44 ± 0.01	10.77 ± 0.04	4.18 ± 0.00	19.38 ± 0.01
UF	2	4	0.51 ± 0.00	12.16 ± 0.01	9.72 ± 0.07	4.19 ± 0.00	17.76 ± 0.01
UF	3	1	0.54 ± 0.00	13.61 ± 0.01	10.89 ± 0.01	4.18 ± 0.04	19.29 ± 0.03

Table 14; continued

Sample	Block	Treatment	Fat	Protein	Casein	Lactose	TS
UF	3	2	0.51 ± 0.00	12.25 ± 0.01	9.81 ± 0.00	4.22 ± 0.00	17.88 ± 0.01
UF	3	3	0.51 ± 0.00	12.24 ± 0.01	9.85 ± 0.03	4.22 ± 0.01	17.85 ± 0.00
UF	3	4	0.52 ± 0.01	12.64 ± 0.02	10.14 ± 0.01	4.19 ± 0.00	16.41 ± 0.03
DF1	1	1	0.91 ± 0.00	13.69 ± 0.00	10.19 ± 0.01	1.04 ± 0.00	16.41 ± 0.03
DF1	1	2	1.09 ± 0.00	14.03 ± 0.01	10.28 ± 0.01	0.92 ± 0.00	16.86 ± 0.01
DF1	1	3	1.00 ± 0.00	12.44 ± 0.01	9.12 ± 0.01	1.01 ± 0.00	15.29 ± 0.01
DF1	1	4	1.01 ± 0.01	14.14 ± 0.01	10.67 ± 0.01	1.00 ± 0.00	17.07 ± 0.00
DF1	2	1	0.81 ± 0.01	13.58 ± 0.01	9.94 ± 0.00	1.15 ± 0.01	16.19 ± 0.03
DF1	2	2	0.85 ± 0.00	15.36 ± 0.02	11.32 ± 0.01	1.00 ± 0.00	17.91 ± 0.01
DF1	2	3	1.02 ± 0.00	12.64 ± 0.00	9.41 ± 0.02	1.08 ± 0.00	15.52 ± 0.03
DF1	2	4	0.79 ± 0.00	12.88 ± 0.01	9.75 ± 0.01	1.11 ± 0.00	15.56 ± 0.04
DF1	3	1	0.87 ± 0.00	14.80 ± 0.01	10.90 ± 0.01	1.05 ± 0.00	17.38 ± 0.04
DF1	3	2	0.91 ± 0.00	13.69 ± 0.00	10.19 ± 0.04	1.04 ± 0.00	16.41 ± 0.01
DF1	3	3	0.83 ± 0.00	14.06 ± 0.01	10.68 ± 0.03	1.10 ± 0.00	16.76 ± 0.01
DF1	3	4	0.79 ± 0.00	12.94 ± 0.02	9.81 ± 0.01	1.04 ± 0.01	15.57 ± 0.01
DF2	1	1	1.06 ± 0.00	12.63 ± 0.01	8.75 ± 0.00	0.64 ± 0.00	14.86 ± 0.03
DF2	1	2	0.95 ± 0.00	14.03 ± 0.00	10.23 ± 0.00	0.59 ± 0.00	16.27 ± 0.00
DF2	1	3	1.07 ± 0.00	13.17 ± 0.01	9.58 ± 0.01	0.56 ± 0.01	15.62 ± 0.01
DF2	1	4	1.03 ± 0.00	13.93 ± 0.00	10.31 ± 0.00	0.51 ± 0.00	16.39 ± 0.01
DF2	2	1	0.91 ± 0.00	15.33 ± 0.01	11.09 ± 0.00	0.67 ± 0.00	17.43 ± 0.04
DF2	2	2	0.84 ± 0.00	14.12 ± 0.01	10.11 ± 0.01	0.60 ± 0.01	16.15 ± 0.04
DF2	2	3	1.18 ± 0.00	14.62 ± 0.02	10.83 ± 0.00	0.56 ± 0.00	17.11 ± 0.01
DF2	2	4	0.83 ± 0.00	12.97 ± 0.01	9.57 ± 0.00	0.59 ± 0.00	15.15 ± 0.05
DF2	3	1	0.94 ± 0.01	16.18 ± 0.01	11.84 ± 0.01	0.63 ± 0.01	18.31 ± 0.04
DF2	3	2	0.85 ± 0.00	13.44 ± 0.04	9.65 ± 0.01	0.62 ± 0.01	15.52 ± 0.01

Table 14; continued

Sample	Block	Treatment	Fat	Protein	Casein	Lactose	TS
DF2	3	3	0.84 ± 0.00	13.29 ± 0.01	9.75 ± 0.00	0.61 ± 0.00	15.45 ± 0.01
DF2	3	4	0.90 ± 0.00	14.74 ± 0.01	11.07 ± 0.01	0.52 ± 0.01	17.01 ± 0.01
DF3	1	1	0.86 ± 0.00	14.02 ± 0.01	9.85 ± 0.01	0.61 ± 0.00	15.98 ± 0.05
DF3	1	2	0.90 ± 0.00	13.23 ± 0.00	9.53 ± 0.01	0.57 ± 0.00	15.39 ± 0.01
DF3	1	3	1.06 ± 0.00	12.72 ± 0.00	9.12 ± 0.01	0.47 ± 0.01	15.04 ± 0.01
DF3	1	4	1.07 ± 0.00	14.91 ± 0.02	10.78 ± 0.01	0.34 ± 0.01	17.25 ± 0.04
DF3	2	1	0.84 ± 0.01	13.60 ± 0.04	9.59 ± 0.06	0.62 ± 0.00	15.51 ± 0.04
DF3	2	2	0.79 ± 0.00	12.82 ± 0.01	8.96 ± 0.01	0.52 ± 0.00	14.69 ± 0.03
DF3	2	3	1.03 ± 0.00	11.79 ± 0.01	8.47 ± 0.01	0.54 ± 0.00	14.03 ± 0.00
DF3	2	4	0.79 ± 0.00	11.60 ± 0.01	8.43 ± 0.01	0.51 ± 0.00	13.67 ± 0.02
DF3	3	1	0.90 ± 0.00	13.23 ± 0.00	9.53 ± 0.00	0.57 ± 0.00	15.39 ± 0.01
DF3	3	2	0.87 ± 0.00	14.19 ± 0.02	10.20 ± 0.06	0.50 ± 0.01	16.19 ± 0.02
DF3	3	3	0.83 ± 0.00	14.09 ± 0.01	10.67 ± 0.03	1.10 ± 0.00	16.78 ± 0.05
DF3	3	4	0.84 ± 0.00	12.63 ± 0.01	9.24 ± 0.03	0.48 ± 0.00	14.74 ± 0.02

Appendix J: Protein Content as Determined by rapidN

Table 17: Protein content of skim milk and products obtained from UF and DF as determined by rapidN

Sample	Block	Treatment	Protein
Milk	1	1	4.18 ± 0.03
Milk	1	2	3.64 ± 0.04
Milk	1	3	3.75 ± 0.05
Milk	1	4	4.10 ± 0.35
Milk	2	1	3.94 ± 0.03
Milk	2	2	3.57 ± 0.09
Milk	2	3	3.96 ± 0.01
Milk	2	4	4.14 ± 0.19
Milk	3	1	4.31 ± 0.09
Milk	3	2	4.04 ± 0.13
Milk	3	3	4.01 ± 0.11
Milk	3	4	4.06 ± 0.08
UF	1	1	12.52 ± 0.11
UF	1	2	12.75 ± 0.10
UF	1	3	13.86 ± 0.07
UF	1	4	14.13 ± 0.14
UF	2	1	12.39 ± 0.05
UF	2	2	12.72 ± 0.05
UF	2	3	13.27 ± 0.12
UF	2	4	12.48 ± 0.10
UF	3	1	13.97 ± 0.04
UF	3	2	12.50 ± 0.06
UF	3	3	12.57 ± 0.10
UF	3	4	13.08 ± 0.09
DF1	1	1	13.81 ± 1.17
DF1	1	2	14.40 ± -0.03
DF1	1	3	12.53± 0.12
DF1	1	4	15.39 ± 0.26
DF1	2	1	13.66 ± 0.15
DF1	2	2	14.98 ± 0.09
DF1	2	3	12.52 ± 0.08
DF1	2	4	12.96 ± 0.03
DF1	3	1	15.21 ± 0.02
DF1	3	2	14.66 ± 0.03
DF1	3	3	14.38 ± 0.10
DF1	3	4	13.09 ± 0.21

Table 15; continued

Sample	Block	Treatment	Protein
DF2	1	2	14.39 ± 0.19
DF2	1	3	13.48 ± 0.28
DF2	1	4	13.89 ± 0.74
DF2	2	1	15.36 ± 0.06
DF2	2	2	13.96 ± 0.06
DF2	2	3	14.96 ± 0.08
DF2	2	4	13.56 ± 0.23
DF2	3	1	16.52 ± 0.15
DF2	3	2	14.05 ± 0.63
DF2	3	3	13.45 ± 0.06
DF2	3	4	15.06 ± 0.41
DF3	1	1	16.81 ± 0.08
DF3	1	2	13.45 ± 0.26
DF3	1	3	13.67 ± 0.13
DF3	1	4	15.46 ± 0.01
DF3	2	1	13.45 ± 0.08
DF3	2	2	12.83 ± 0.09
DF3	2	3	12.37 ± 0.02
DF3	2	4	12.10 ± 0.02
DF3	3	1	13.72 ± 0.22
DF3	3	2	14.44 ± 0.09
DF3	3	3	14.50 ± 0.24
DF3	3	4	13.01 ± 0.16

Protein in Spray-dried MPC as Determined by rapidN

Table 18: Protein analysis of dry MPC, as determined by Elementar rapidN. Means and standard deviations were calculated from two measurements

Sample	Block	Treatment	Protein
MPC	1	1	84.73 ± 0.26
MPC	1	2	82.42 ± 0.18
MPC	1	3	81.70 ± 0.28
MPC	1	4	81.37 ± 0.19
MPC	2	1	84.90 ± 0.02
MPC	2	2	85.15 ± 0.08
MPC	2	3	81.13 ± 0.71
MPC	2	4	81.46 ± 0.69
MPC	3	1	85.33 ± 0.08
MPC	3	2	84.95 ± 0.23
MPC	3	3	81.93 ± 0.15
MPC	3	4	81.78 ± 0.06

Minitab Output for Protein in Spray-dried MPC as Determined by rapidN

General Linear Model: protein versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for protein, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS  Adj MS      F      P
block       2   1.8243   1.8243  0.9122   1.60  0.278
treatment   3  28.3270  28.3270  9.4423  16.52  0.003
Error       6   3.4304   3.4304  0.5717
Total      11  33.5817


S = 0.756125   R-Sq = 89.79%   R-Sq(adj) = 81.27%


Unusual Observations for protein

Obs  protein      Fit  SE Fit  Residual  St Resid
  2  82.4200  83.6575  0.5347   -1.2375     -2.31 R

R denotes an observation with a large standardized residual.


Least Squares Means for protein
```

```
treatment   Mean
1          84.99
2          84.17
3          81.59
4          81.54


Grouping Information Using Tukey Method and 99.0% Confidence

treatment  N  Mean  Grouping
1          3  85.0  A
2          3  84.2  A B
3          3  81.6    B
4          3  81.5    B

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable protein
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

treatment   Lower  Center    Upper   --+---------+---------+---------+----
2          -3.882  -0.813   2.2556            (---------*----------)
3          -6.469  -3.400  -0.3311   (----------*---------)
4          -6.519  -3.450  -0.3811   (---------*----------)
                                     --+---------+---------+---------+----
                                    -6.0      -3.0       0.0       3.0


treatment = 2  subtracted from:

treatment   Lower  Center   Upper   --+---------+---------+---------+----
3          -5.656  -2.587  0.4823      (---------*----------)
4          -5.706  -2.637  0.4323      (---------*---------)
                                    --+---------+---------+---------+----
                                   -6.0      -3.0       0.0       3.0


treatment = 3  subtracted from:

treatment   Lower    Center  Upper   --+---------+---------+---------+----
4          -3.119  -0.05000  3.019              (---------*---------)
                                     --+---------+---------+---------+----
                                    -6.0      -3.0       0.0       3.0


Tukey Simultaneous Tests
Response Variable protein
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
2              -0.813      0.6174   -1.317    0.5857
3              -3.400      0.6174   -5.507    0.0061
4              -3.450      0.6174   -5.588    0.0056


treatment = 2  subtracted from:

           Difference       SE of           Adjusted
```

treatment	of Means	Difference	T-Value	P-Value
3	-2.587	0.6174	-4.190	0.0223
4	-2.637	0.6174	-4.271	0.0204

treatment = 3 subtracted from:

treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	-0.05000	0.6174	-0.08099	0.9998

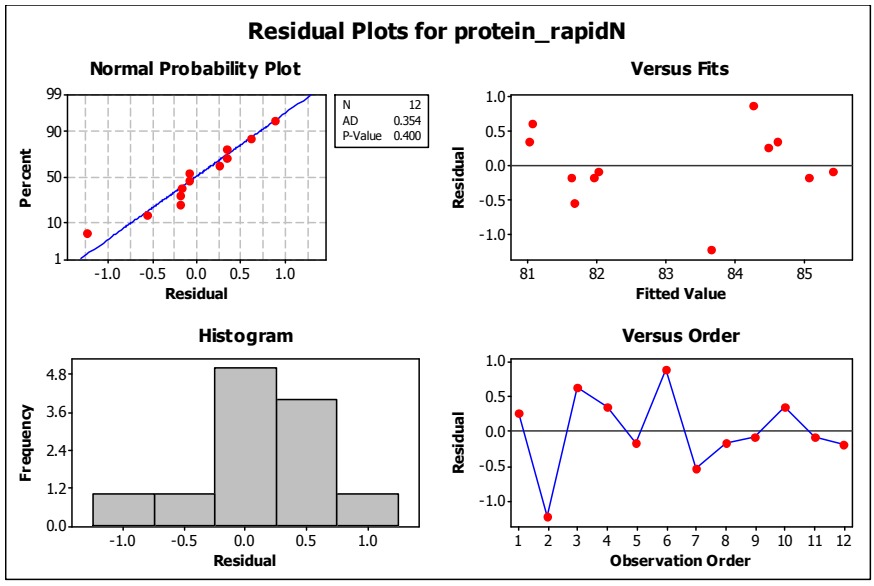


Figure 69: Residual plots for protein of dry MPC as determined by Elementar rapid N; Top Left: Normal probability plot (p = 0.400); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

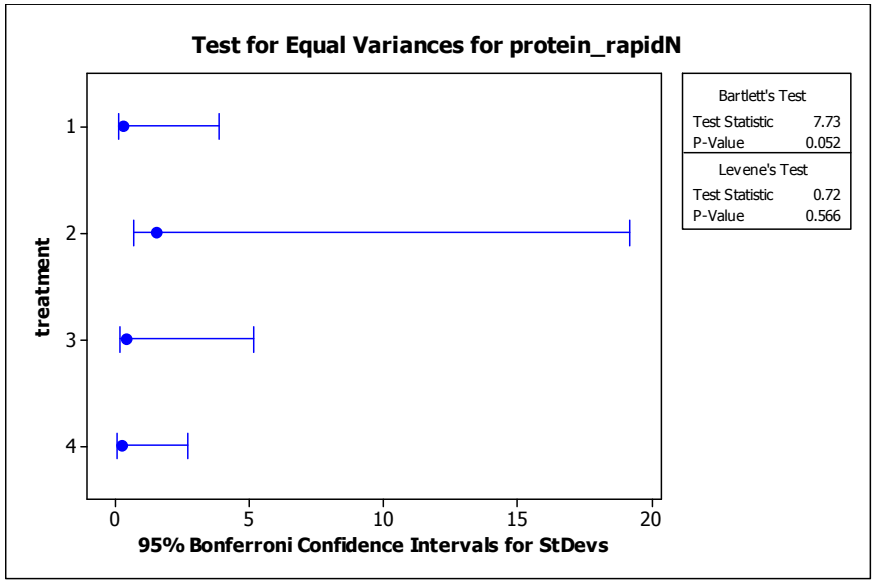


Figure 70: Plot of Variance for protein of dry MPC as determined by Elementar rapid N; Bartlett's Test statistic = 7.73, p-value = 0.052; Levene's Test statistic = 0.72, p-value = 0.566

Appendix K: Comparison of FOSS Milkoscan FT2 and rapidN in Determination of Protein Content of Products Obtained from UF/DF Processes

The Mixed Procedure

Model Information	
Data Set	GUALCO.FT2_RN
Dependent Variable	protein
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Prasad-Rao-Jeske-Kackar-Harville
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	**Levels**	**Values**
process	5	DF1 DF2 DF3 Milk UF
sample	24	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
block	3	1 2 3
treatment	4	1 2 3 4
test	2	1 2

Dimensions	
Covariance Parameters	4
Columns in X	90
Columns in Z	75

Dimensions	
Subjects	1
Max Obs Per Subject	120

Number of Observations	
Number of Observations Read	120
Number of Observations Used	120
Number of Observations Not Used	0

Iteration History			
Iteration	**Evaluations**	**-2 Res Log Like**	**Criterion**
0	1	255.83107178	
1	1	197.99255796	0.00000000

Convergence criteria met.

Covariance Parameter Estimates	
Cov Parm	**Estimate**
block	0.003187
block*treatment	0.04540
proces*block*treatme	0.6742
Residual	0.1048

Fit Statistics	
-2 Res Log Likelihood	198.0
AIC (smaller is better)	206.0
AICC (smaller is better)	206.5
BIC (smaller is better)	202.4

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
treatment	3	6	0.94	0.4778
process	4	32	327.81	<.0001
process*treatment	12	32	0.54	0.8748
test	1	40	41.05	<.0001
treatment*test	3	40	0.89	0.4570
process*test	4	40	1.78	0.1514
process*treatme*test	12	40	0.72	0.7219

Appendix L: Ca, Mg, K, Na Analysis of Skim Milk, UF/DF Products (Wet Basis)

Table 19: Minerals analysis (Ca, Mg, K, and Na) of skim milk and all products obtained by UF and DF, as determined by ICP-MS (wet basis)

Sample	Block	Treatment	Ca mg/L	Mg mg/L	K mg/L	Na mg/L
Milk	1	1	1113.0	85.1	1368.0	393.4
Milk	1	2	1137.0	87.8	1468.0	402.7
Milk	1	3	1041.0	83.6	1324.0	383.4
Milk	1	4	1088.0	80.1	1282.0	360.9
Milk	2	1	1224.0	96.0	1560.0	438.0
Milk	2	2	1251.0	110.1	1696.0	491.5
Milk	2	3	1156.0	84.9	1460.0	398.9
Milk	2	4	1551.0	84.9	1950.0	651.2
Milk	3	1	1138.0	84.8	1376.0	417.6
Milk	3	2	1191.0	91.3	1517.0	469.7
Milk	3	3	1465.0	112.7	1807.0	601.9
Milk	3	4	1092.0	85.5	1362.0	407.0
UF	1	1	4123.0	204.6	1682.0	536.4
UF	1	2	3551.0	170.6	1559.0	423.1
UF	1	3	3242.0	180.7	1534.0	439.8
UF	1	4	3544.0	157.5	1432.0	384.2
UF	2	1	3837.0	187.7	1611.0	467.8
UF	2	2	3951.0	194.5	1764.0	486.6
UF	2	3	3911.0	186.7	1530.0	444.4
UF	2	4	4090.0	202.0	1730.0	517.0
UF	3	1	3749.0	176.4	1419.0	432.5
UF	3	2	3721.0	187.1	1775.0	469.7
UF	3	3	3273.0	154.4	1432.0	456.6
UF	3	4	3552.0	179.3	1552.0	462.2
DF1	1	1	4035.0	203.8	255.2	181.7
DF1	1	2	2964.0	146.9	211.1	1643.0
DF1	1	3	3242.0	151.0	234.7	1962.0
DF1	1	4	3230.0	139.8	209.9	2457.0
DF1	2	1	3811.0	205.6	364.1	209.5
DF1	2	2	4088.0	188.9	245.3	1086.0
DF1	2	3	3461.0	170.3	259.1	2014.0
DF1	2	4	3504.0	171.7	259.9	2867.0
DF1	3	1	3833.0	206.2	316.8	120.9
DF1	3	2	3310.0	164.0	273.8	559.0
DF1	3	3	3326.0	159.0	248.9	1784.0
DF1	3	4	3547.0	165.6	236.0	282.0

Table 17, continued

Sample	Block	Treatment	Ca mg/L	Mg mg/L	K mg/L	Na mg/L
DF2	1	1	3252.0	209.9	26.6	116.3
DF2	1	2	3531.0	196.5	9.5	1144.0
DF2	1	3	3051.0	164.8	25.0	1987.0
DF2	1	4	2794.0	142.8	6.9	2680.0
DF2	2	1	4363.0	286.1	55.2	149.2
DF2	2	2	3591.0	200.4	19.2	1277.0
DF2	2	3	3622.0	177.8	8.8	2196.0
DF2	2	4	3151.0	166.2	17.1	3125.0
DF2	3	1	4294.0	274.3	85.3	118.3
DF2	3	2	3256.0	181.2	25.9	963.3
DF2	3	3	2897.0	164.3	6.8	2024.0
DF2	3	4	3181.0	165.9	ND	2757.0
DF3	1	1	3490.0	249.7	ND	54.7
DF3	1	2	2699.0	176.6	ND	2176.0
DF3	1	3	2911.0	175.4	ND	2297.0
DF3	1	4	3016.0	171.1	ND	2926.0
DF3	2	1	3781.0	295.4	ND	61.9
DF3	2	2	2960.0	206.6	ND	1109.0
DF3	2	3	2654.0	167.4	ND	1993.0
DF3	2	4	2795.0	178.4	ND	3591.0
DF3	3	1	4333.0	313.3	ND	71.2
DF3	3	2	3724.0	233.0	ND	1313.0
DF3	3	3	3595.0	210.7	ND	2024.0
DF3	3	4	2802.0	165.8	ND	3228.0

PROC Mixed to Determine Differences Between DF1, DF2, DF3 Processes and UF Process

Ca Difference Between DF1, DF2, DF3 Processes and UF Process

The Mixed Procedure

Model Information	
Data Set	GUALCO.MPC4
Dependent Variable	DIFF_Ca
Covariance Structures	Variance Components, Autoregressive
Subject Effects	Sample, Sample
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based

Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
Process	3	DF1 DF2 DF3
Sample	12	1 2 3 4 5 6 7 8 9 10 11 12
Block	3	1 2 3
Treatment	4	1 2 3 4

Dimensions	
Covariance Parameters	3
Columns in X	2 0
Columns in Z Per Subject	3
Subjects	1 2
Max Obs Per Subject	3

Number of Observations	
Number of Observations Read	3 6
Number of Observations Used	3 6
Number of Observations Not Used	0

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	375.81066595	
1	2	374.04996866	0.00000001
2	1	374.04996658	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	Subject	Estimate
Block	Sample	60779
AR(1)	Sample	0.001135

Residual		152766

Fit Statistics	
-2 Res Log Likelihood	374.0
AIC (smaller is better)	380.0
AICC (smaller is better)	381.2
BIC (smaller is better)	381.5

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Process	2	16	1.79	0.1985
Treatment	3	16	1.80	0.1875
Process*Treatment	6	16	0.33	0.9137

K Difference Between DF1, DF2, DF3 Processes and UF Process
The Mixed Procedure

Model Information	
Data Set	GUALCO.MPC4
Dependent Variable	DIFF_K
Covariance Structures	Variance Components, Autoregressive
Subject Effects	Sample, Sample
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
Process	3	DF1 DF2 DF3
Sample	12	1 2 3 4 5 6 7 8 9 10 11 12
Block	3	1 2 3
Treatment	4	1 2 3 4

Dimensions	
Covariance Parameters	3
Columns in X	20
Columns in Z Per Subject	3
Subjects	12
Max Obs Per Subject	3

Number of Observations	
Number of Observations Read	36
Number of Observations Used	36
Number of Observations Not Used	0

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	311.72410402	
1	2	275.69049709	2059.6268490
2	1	270.87053030	500.73405620
3	1	266.57830772	113.77264947
4	1	262.90179901	22.23647227
5	2	259.73026528	1.14031119
6	2	259.47831939	0.19884516
7	2	259.42850547	0.00138714
8	3	259.40625178	.
9	1	259.40577828	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	Subject	Estimate
Block	Sample	0
AR(1)	Sample	0.9797
Residual		14255

Fit Statistics

-2 Res Log Likelihood	259.4
AIC (smaller is better)	263.4
AICC (smaller is better)	264.0
BIC (smaller is better)	264.4

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Process	2	16	583.72	<.0001
Treatment	3	16	1.63	0.2220
Process*Treatment	6	16	1.76	0.1719

Mg Difference Between DF1, DF2, DF3 Processes and UF Process
The Mixed Procedure

Model Information	
Data Set	GUALCO.MPC4
Dependent Variable	DIFF_Mg
Covariance Structures	Variance Components, Autoregressive
Subject Effects	Sample, Sample
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
Process	3	DF1 DF2 DF3
Sample	12	1 2 3 4 5 6 7 8 9 10 11 12
Block	3	1 2 3
Treatment	4	1 2 3 4

Dimensions	
Covariance Parameters	3
Columns in X	2

	0
Columns in Z Per Subject	3
Subjects	1 2
Max Obs Per Subject	3

Number of Observations	
Number of Observations Read	36
Number of Observations Used	36
Number of Observations Not Used	0

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	239.73138362	
1	3	231.76721108	0.00004451
2	1	231.76299899	0.00000013
3	1	231.76298647	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	Subject	Estimate
Block	Sample	321.61
AR(1)	Sample	0.2950
Residual		405.25

Fit Statistics	
-2 Res Log Likelihood	231.8
AIC (smaller is better)	237.8
AICC (smaller is better)	239.0
BIC (smaller is better)	239.2

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Process	2	16	12.52	0.0005
Treatment	3	16	6.52	0.0043
Process*Treatment	6	16	2.08	0.1136

Least Squares Means							
Effect	Process	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Process	DF1		-9.0583	7.7828	16	-1.16	0.2615
Process	DF2		12.3917	7.7828	16	1.59	0.1309
Process	DF3		30.1583	7.7828	16	3.87	0.0013
Treatment		1	59.8000	13.1362	16	4.55	0.0003
Treatment		2	4.1667	13.1362	16	0.32	0.7552
Treatment		3	-2.7444	13.1362	16	-0.21	0.8371
Treatment		4	-16.5667	13.1362	16	-1.26	0.2253

Differences of Least Squares Means										
Effect	Process	Treatment	Process	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P
Process	DF1		DF2		-21.4500	6.9003	16	-3.11	0.0068	Tukey-Kramer	0.0175
Process	DF1		DF3		-39.2167	7.8526	16	-4.99	0.0001	Tukey-Kramer	0.0004
Process	DF2		DF3		-17.7667	6.9003	16	-2.57	0.02	Tukey-Kramer	0.0505

									04		
Treatment		1		2	55.6333	18.5774	16	2.99	0.00086	Tukey	0.0386
Treatment		1		3	62.5444	18.5774	16	3.37	0.00039	Tukey	0.0185
Treatment		1		4	76.3667	18.5774	16	4.11	0.00008	Tukey	0.0041
Treatment		2		3	6.9111	18.5774	16	0.37	0.71448	Tukey	0.9818
Treatment		2		4	20.7333	18.5774	16	1.12	0.28089	Tukey	0.6852
Treatment		3		4	13.8222	18.5774	16	0.74	0.46776	Tukey	0.8778

Na Difference Between DF1, DF2, DF3 Processes and UF Process
The Mixed Procedure

Model Information	
Data Set	GUALCO.MPC4
Dependent Variable	DIFF_Na
Covariance Structures	Variance Components, Unstructured
Subject Effects	Sample, Sample
Estimation Method	REML
Residual Variance Method	None

Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	Levels	Values
Process	3	DF1 DF2 DF3
Sample	12	1 2 3 4 5 6 7 8 9 10 11 12
Block	3	1 2 3
Treatment	4	1 2 3 4

Dimensions	
Covariance Parameters	7
Columns in X	20
Columns in Z Per Subject	3
Subjects	12
Max Obs Per Subject	3

Number of Observations	
Number of Observations Read	36
Number of Observations Used	36
Number of Observations Not Used	0

Iteration History			
Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	377.95673596	
1	1	355.29144012	0.00000000

Convergence criteria met but final hessian is not positive definite.

Covariance Parameter Estimates		
Cov Parm	Subject	Estimate
Block	Sample	11388
UN(1,1)	Sample	554907

UN(2,1)	Sample	43236
UN(2,2)	Sample	6853.40
UN(3,1)	Sample	75580
UN(3,2)	Sample	0
UN(3,3)	Sample	104628

Fit Statistics	
-2 Res Log Likelihood	355.3
AIC (smaller is better)	369.3
AICC (smaller is better)	376.3
BIC (smaller is better)	372.7

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Process	2	16	3.57	0.0523
Treatment	3	16	32.76	<.0001
Process*Treatment	6	16	1.70	0.1849

Least Squares Means						
Effect	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|
Treatment	1	-358.49	193.08	16	-1.86	0.0819
Treatment	2	792.46	193.08	16	4.10	0.0008
Treatment	3	1584.29	193.08	16	8.21	<.0001
Treatment	4	2202.53	193.08	16	11.41	<.0001

Differences of Least Squares Means									
Effect	Treatment	Treatment	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment	Adj P

Treatment	1	2	-1150.94	273.05	16	-4.22	0.0007	Tukey	0.0033
Treatment	1	3	-1942.78	273.05	16	-7.12	<.0001	Tukey	<.0001
Treatment	1	4	-2561.02	273.05	16	-9.38	<.0001	Tukey	<.0001
Treatment	2	3	-791.83	273.05	16	-2.90	0.0104	Tukey	0.0464
Treatment	2	4	-1410.08	273.05	16	-5.16	<.0001	Tukey	0.0005
Treatment	3	4	-618.24	273.05	16	-2.26	0.0378	Tukey	0.1484

Appendix M: Ca, Mg, K, and Na Analysis of Skim Milk, UF/DF Products (Dry Basis)

Ca Difference Between DF1, DF2, DF3 Processes and UF Process

The Mixed Procedure

Model Information	
Data Set	WORK.DRY_BASIS
Dependent Variable	DIFF_ca
Covariance Structures	Variance Components, Autoregressive
Subject Effects	sample, sample
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	**Levels**	**Values**
process	3	DF1 DF2 DF3
sample	12	1 2 3 4 5 6 7 8 9 10 11 12
block	3	1 2 3
treatment	4	1 2 3 4

Dimensions	
Covariance Parameters	3
Columns in X	20
Columns in Z Per Subject	3

Dimensions	
Subjects	12
Max Obs Per Subject	3

Number of Observations	
Number of Observations Read	36
Number of Observations Used	36
Number of Observations Not Used	0

Iteration History			
Iteration	**Evaluations**	**-2 Res Log Like**	**Criterion**
0	1	3.95812742	
1	4	2.02016066	0.00031425
2	1	2.01363299	0.00000012
3	1	2.01363056	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
block	sample	1.89E-19
AR(1)	sample	0.3722
Residual		0.04061

Fit Statistics	
-2 Res Log Likelihood	2.0
AIC (smaller is better)	6.0
AICC (smaller is better)	6.6
BIC (smaller is better)	7.0

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
process	2	16	0.43	0.6547
treatment	3	16	4.92	0.0131
process*treatment	6	16	1.23	0.3431

Least Squares Means							
Effect	**process**	**treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
process	DF1		0.1277	0.05817	16	2.19	0.0433
process	DF2		0.06897	0.05817	16	1.19	0.2531
process	DF3		0.07241	0.05817	16	1.24	0.2311
treatment		1	0.2899	0.08466	16	3.42	0.0035
treatment		2	0.01288	0.08466	16	0.15	0.8810
treatment		3	0.1894	0.08466	16	2.24	0.0399
treatment		4	-0.1334	0.08466	16	-1.58	0.1346

Differences of Least Squares Means											
Effect	**process**	**treatment**	**_process**	**_treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**	**Adj P**
process	DF1		DF2		0.05871	0.06518	16	0.90	0.3811	Tukey-Kramer	0.6476
process	DF1		DF3		0.05526	0.07635	16	0.72	0.4796	Tukey-Kramer	0.7532
process	DF2		DF3		-0.00345	0.06518	16	-0.05	0.9585	Tukey-Kramer	0.9985
treatment		1		2	0.2770	0.1197	16	2.31	0.0343	Tukey	0.1363
treatment		1		3	0.1005	0.1197	16	0.84	0.4135	Tukey	0.8349
treatment		1		4	0.4233	0.1197	16	3.54	0.0027	Tukey	0.0132
treatment		2		3	-0.1765	0.1197	16	-1.47	0.1599	Tukey	0.4748
treatment		2		4	0.1463	0.1197	16	1.22	0.2394	Tukey	0.6224
treatment		3		4	0.3228	0.1197	16	2.70	0.0159	Tukey	0.0683

Na Difference Between DF1, DF2, DF3 Processes and UF Process

The Mixed Procedure

Model Information	
Data Set	WORK.DRY_BASIS
Dependent Variable	DIFF_mg
Covariance Structures	Variance Components, Autoregressive
Subject Effects	sample, sample
Estimation Method	REML
Residual Variance Method	Profile

Model Information	
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	**Levels**	**Values**
process	3	DF1 DF2 DF3
sample	12	1 2 3 4 5 6 7 8 9 10 11 12
block	3	1 2 3
treatment	4	1 2 3 4

Dimensions	
Covariance Parameters	3
Columns in X	20
Columns in Z Per Subject	3
Subjects	12
Max Obs Per Subject	3

Number of Observations	
Number of Observations Read	36
Number of Observations Used	36
Number of Observations Not Used	0

Iteration History			
Iteration	**Evaluations**	**-2 Res Log Like**	**Criterion**
0	1	-4.33208256	
1	2	-90.43885417	0.43609749
2	1	-90.53165496	0.00006094
3	1	-90.53554938	0.00000573
4	1	-90.53593703	0.00000001

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
block	sample	0.02759
AR(1)	sample	0.8344
Residual		0.000393

Fit Statistics	
-2 Res Log Likelihood	-90.5
AIC (smaller is better)	-84.5
AICC (smaller is better)	-83.3
BIC (smaller is better)	-83.1

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
process	2	16	27.00	<.0001
treatment	3	16	1.55	0.2393
process*treatment	6	16	3.42	0.0228

Least Squares Means							
Effect	**process**	**treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
process	DF1		-1.9197	0.04829	16	-39.75	<.0001
process	DF2		-1.9062	0.04829	16	-39.47	<.0001
process	DF3		-1.8873	0.04829	16	-39.08	<.0001
treatment		1	-1.9453	0.09649	16	-20.16	<.0001
treatment		2	-1.9388	0.09649	16	-20.09	<.0001

Least Squares Means							
Effect	**process**	**treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
treatment		3	-1.7294	0.09649	16	-17.92	<.0001
treatment		4	-2.0041	0.09649	16	-20.77	<.0001

Differences of Least Squares Means											
Effect	**process**	**treatment**	**_process**	**_treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**	**Adj P**
process	DF1		DF2		-0.01355	0.003293	16	-4.11	0.0008	Tukey-Kramer	0.0022
process	DF1		DF3		-0.03241	0.004461	16	-7.27	<.0001	Tukey-Kramer	<.0001
process	DF2		DF3		-0.01886	0.003293	16	-5.73	<.0001	Tukey-Kramer	<.0001
treatment		1		2	-0.00654	0.1365	16	-0.05	0.9624	Tukey	1.0000
treatment		1		3	-0.2159	0.1365	16	-1.58	0.1332	Tukey	0.4156
treatment		1		4	0.05878	0.1365	16	0.43	0.6724	Tukey	0.9723
treatment		2		3	-0.2094	0.1365	16	-1.53	0.1445	Tukey	0.4414
treatment		2		4	0.06532	0.1365	16	0.48	0.6386	Tukey	0.9627
treatment		3		4	0.2747	0.1365	16	2.01	0.0613	Tukey	0.2243

K Difference Between DF1, DF2, DF3 Processes and UF Process

The Mixed Procedure

Model Information	
Data Set	WORK.DRY_BASIS
Dependent Variable	DIFF_k
Covariance Structure	Variance Components
Subject Effects	sample, sample
Estimation Method	REML
Residual Variance Method	Parameter
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	**Levels**	**Values**
process	3	DF1 DF2 DF3
sample	12	1 2 3 4 5 6 7 8 9 10 11 12
block	3	1 2 3
treatment	4	1 2 3 4

Dimensions	
Covariance Parameters	2
Columns in X	20
Columns in Z Per Subject	3
Subjects	12
Max Obs Per Subject	3

Number of Observations	
Number of Observations Read	36
Number of Observations Used	36
Number of Observations Not Used	0

Iteration History			
Iteration	**Evaluations**	**-2 Res Log Like**	**Criterion**
0	1	-3.43961829	
1	1	-75.53850632	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
block	sample	0.02910
process	sample	0.000187

Fit Statistics	
-2 Res Log Likelihood	-75.5
AIC (smaller is better)	-71.5
AICC (smaller is better)	-71.0
BIC (smaller is better)	-70.6

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
process	2	16	493.11	<.0001
treatment	3	16	1.60	0.2293
process*treatment	6	16	1.75	0.1737

Least Squares Means							
Effect	**process**	**treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
process	DF1		-1.8665	0.04941	16	-37.78	<.0001
process	DF2		-2.0108	0.04941	16	-40.70	<.0001
process	DF3		-2.0251	0.04941	16	-40.99	<.0001
treatment		1	-2.0249	0.09860	16	-20.54	<.0001
treatment		2	-2.0043	0.09860	16	-20.33	<.0001

Least Squares Means							
Effect	**process**	**treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
treatment		3	-1.7834	0.09860	16	-18.09	<.0001
treatment		4	-2.0572	0.09860	16	-20.86	<.0001

Differences of Least Squares Means											
Effect	**process**	**treatment**	**_process**	**_treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**	**Adj P**
process	DF1		DF2		0.1442	0.005584	16	25.83	<.0001	Tukey-Kramer	<.0001
process	DF1		DF3		0.1585	0.005584	16	28.39	<.0001	Tukey-Kramer	<.0001
process	DF2		DF3		0.01430	0.005584	16	2.56	0.0209	Tukey-Kramer	0.0519
treatment		1		2	-0.02059	0.1394	16	-0.15	0.8845	Tukey	0.9988
treatment		1		3	-0.2415	0.1394	16	-1.73	0.1025	Tukey	0.3404
treatment		1		4	0.03229	0.1394	16	0.23	0.8198	Tukey	0.9954
treatment		2		3	-0.2209	0.1394	16	-1.58	0.1327	Tukey	0.4146
treatment		2		4	0.05288	0.1394	16	0.38	0.7095	Tukey	0.9808
treatment		3		4	0.2738	0.1394	16	1.96	0.0672	Tukey	0.2423

Na Difference Between DF1, DF2, DF3 Processes and UF Process

The Mixed Procedure

Model Information	
Data Set	WORK.DRY_BASIS
Dependent Variable	DIFF_na
Covariance Structure	Variance Components
Subject Effects	sample, sample
Estimation Method	REML
Residual Variance Method	Parameter
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information		
Class	**Levels**	**Values**
process	3	DF1 DF2 DF3
sample	12	1 2 3 4 5 6 7 8 9 10 11 12
block	3	1 2 3
treatment	4	1 2 3 4

Dimensions	
Covariance Parameters	2
Columns in X	20
Columns in Z Per Subject	3
Subjects	12
Max Obs Per Subject	3

Number of Observations	
Number of Observations Read	36
Number of Observations Used	36
Number of Observations Not Used	0

Iteration History			
Iteration	**Evaluations**	**-2 Res Log Like**	**Criterion**
0	1	31.64004511	
1	1	27.59411495	0.00000000

Convergence criteria met.

Covariance Parameter Estimates		
Cov Parm	**Subject**	**Estimate**
block	sample	0.05443
process	sample	0.07190

Fit Statistics	
-2 Res Log Likelihood	27.6
AIC (smaller is better)	31.6
AICC (smaller is better)	32.2
BIC (smaller is better)	32.6

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
process	2	16	5.68	0.0137
treatment	3	16	21.31	<.0001
process*treatment	6	16	2.36	0.0794

Least Squares Means							
Effect	**process**	**treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
process	DF1		-1.2468	0.1026	16	-12.15	<.0001
process	DF2		-1.0645	0.1026	16	-10.37	<.0001
process	DF3		-0.8778	0.1026	16	-8.56	<.0001
treatment		1	-2.0249	0.1617	16	-12.53	<.0001
treatment		2	-1.2763	0.1617	16	-7.89	<.0001
treatment		3	-0.5423	0.1617	16	-3.35	0.0040
treatment		4	-0.4086	0.1617	16	-2.53	0.0224

Differences of Least Squares Means											
Effect	**process**	**treatment**	**_process**	**_treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**	**Adj P**
process	DF1		DF2		-0.1823	0.1095	16	-1.67	0.1153	Tukey-Kramer	0.2485
process	DF1		DF3		-0.3690	0.1095	16	-3.37	0.0039	Tukey-Kramer	0.0103
process	DF2		DF3		-0.1867	0.1095	16	-1.71	0.1075	Tukey-Kramer	0.2337
treatment		1		2	-0.7487	0.2286	16	-3.27	0.0048	Tukey	0.0222
treatment		1		3	-1.4827	0.2286	16	-6.49	<.0001	Tukey	<.0001
treatment		1		4	-1.6164	0.2286	16	-7.07	<.0001	Tukey	<.0001
treatment		2		3	-0.7340	0.2286	16	-3.21	0.0055	Tukey	0.0253
treatment		2		4	-0.8677	0.2286	16	-3.80	0.0016	Tukey	0.0078
treatment		3		4	-0.1337	0.2286	16	-0.58	0.5668	Tukey	0.9352

General Regression Analysis: CaMgRatio versus conc-75, (conc-75)2, Block

Regression Equation

Block
1 CaMgRatio = 16.3193 + 0.021788 conc-75 - 0.000143511 (conc-75)2

2 CaMgRatio = 15.1105 + 0.021788 conc-75 - 0.000143511 (conc-75)2

3 CaMgRatio = 16.3922 + 0.021788 conc-75 - 0.000143511 (conc-75)2

Coefficients

Term	Coef	SE Coef	T	P
Constant	15.9407	0.160372	99.3979	0.000
conc-75	0.0218	0.001792	12.1575	0.000
(conc-75)2	-0.0001	0.000040	-3.5812	0.009
Block				
1	0.3786	0.141681	2.6724	0.032
2	-0.8302	0.141681	-5.8596	0.001

Summary of Model

S = 0.347047 R-Sq = 96.54% R-Sq(adj) = 94.56%
PRESS = 2.61286 R-Sq(pred) = 89.26%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	4	23.4925	23.4925	5.8731	48.763	0.0000339
conc-75	1	17.8019	17.8019	17.8019	147.806	0.0000058
(conc-75)2	1	1.5447	1.5447	1.5447	12.825	0.0089619
Block	2	4.1459	4.1459	2.0730	17.211	0.0019838
Error	7	0.8431	0.8431	0.1204		
Total	11	24.3356				

Fits and Diagnostics for Unusual Observations

No unusual observations

Appendix N: Mineral Content of Dry MPC

Table 20: Mineral analysis of dry MPC, as determined by ICP-MS. Means are shown and were calculated from three measurements, ND (not detected) values were below the instrument detection limit (0.1 mg/g) and were assumed to be 0 in the statistical analysis.

Sample	Block	Treatment	Ca mg/g	Mg mg/g	K mg/g	Na mg/g
Pdr	1	1	8.02	1.567	ND	ND
Pdr	1	2	8.384	1.311	ND	5.97
Pdr	1	3	6.309	0.9886	ND	9.86
Pdr	1	4	6.594	0.9383	ND	13.32
Pdr	2	1	9.69	1.845	ND	ND
Pdr	2	2	7.579	1.291	ND	5.174
Pdr	2	3	6.732	0.9169	ND	10.35
Pdr	2	4	5.818	0.811	ND	14.16
Pdr	3	1	7.987	1.788	ND	ND
Pdr	3	2	7.515	1.142	ND	6.21
Pdr	3	3	6.87	0.9631	ND	10.74
Pdr	3	4	6.19	0.90	ND	15.03

Minitab Output for Mineral Content of Dry MPC

General Linear Model: Ca, Mg, Na versus block, treatment

```
Factor     Type   Levels  Values
block      fixed       3  1, 2, 3
treatment  fixed       4  1, 2, 3, 4


Analysis of Variance for Ca, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS  Adj MS     F      P
block       2   0.1998   0.1998  0.0999  0.23  0.803
treatment   3  10.5794  10.5794  3.5265  8.02  0.016
Error       6   2.6382   2.6382  0.4397
Total      11  13.4174


S = 0.663101   R-Sq = 80.34%   R-Sq(adj) = 63.95%


Unusual Observations for Ca

Obs       Ca      Fit   SE Fit  Residual  St Resid
  5  9.69000  8.71308  0.46888   0.97692      2.08 R

R denotes an observation with a large standardized residual.


Analysis of Variance for Mg, using Adjusted SS for Tests
```

Source	DF	Seq SS	Adj SS	Adj MS	F	P
block	2	0.00072	0.00072	0.00036	0.03	0.970
treatment	3	1.33952	1.33952	0.44651	37.93	0.000
Error	6	0.07063	0.07063	0.01177		
Total	11	1.41087				

S = 0.108499 R-Sq = 94.99% R-Sq(adj) = 90.82%

Unusual Observations for Mg

Obs	Mg	Fit	SE Fit	Residual	St Resid
1	1.56700	1.72940	0.07672	-0.16240	-2.12 R

R denotes an observation with a large standardized residual.

Analysis of Variance for Na, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
block	2	1.132	1.132	0.566	2.59	0.154
treatment	3	334.823	334.823	111.608	511.21	0.000
Error	6	1.310	1.310	0.218		
Total	11	337.265				

S = 0.467247 R-Sq = 99.61% R-Sq(adj) = 99.29%

MANOVA for block
s = 2 m = 0.0 n = 1.0

Criterion	Test Statistic	F	DF Num	DF Denom	P
Wilks'	0.34646	0.932	6	8	0.521
Lawley-Hotelling	1.84896	0.924	6	6	0.537
Pillai's	0.66650	0.833	6	10	0.571
Roy's	1.82851				

SSCP Matrix (adjusted) for block

	Ca	Mg	Na
Ca	0.1998	0.01067	-0.3939
Mg	0.0107	0.00072	-0.0137
Na	-0.3939	-0.01373	1.1325

SSCP Matrix (adjusted) for Error

	Ca	Mg	Na
Ca	2.6382	0.26788	0.43134
Mg	0.2679	0.07063	-0.09092
Na	0.4313	-0.09092	1.30992

Partial Correlations for the Error SSCP Matrix

	Ca	Mg	Na
Ca	1.00000	0.62056	0.23203
Mg	0.62056	1.00000	-0.29889
Na	0.23203	-0.29889	1.00000

EIGEN Analysis for block

Eigenvalue	1.8285	0.02045	0.00000
Proportion	0.9889	0.01106	0.00000
Cumulative	0.9889	1.00000	1.00000

Eigenvector	1	2	3
Ca	-0.6681	0.5204	-0.4218
Mg	3.7773	0.5274	4.4931
Na	1.0819	0.1952	-0.0922

MANOVA for treatment
s = 3 m = -0.5 n = 1.0

	Test		DF		
Criterion	Statistic	Approx F	Num	Denom	P
Wilks'	0.00089	18.550	9	9	0.000
Lawley-Hotelling	345.98873	102.515	9	8	0.000
Pillai's	1.74204	2.770	9	18	0.031
Roy's	343.92102				

SSCP Matrix (adjusted) for treatment

	Ca	Mg	Na
Ca	10.58	3.63	-58.79
Mg	3.63	1.34	-20.65
Na	-58.79	-20.65	334.82

EIGEN Analysis for treatment

Eigenvalue	343.921	1.980	0.088
Proportion	0.994	0.006	0.000
Cumulative	0.994	1.000	1.000

Eigenvector	1	2	3
Ca	-0.4793	0.581	0.573
Mg	2.3445	-5.403	0.207
Na	1.0733	-0.229	0.113

General Linear Model: Na versus block, treatment

Factor	Type	Levels	Values
block	random	3	1, 2, 3
treatment	fixed	4	1, 2, 3, 4

Analysis of Variance for Na, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
block	2	0.821	0.821	0.411	1.10	0.391
treatment	3	235.332	235.332	78.444	210.83	0.000

```
Error       6    2.232    2.232   0.372
Total      11  238.386


S = 0.609975   R-Sq = 99.06%   R-Sq(adj) = 98.28%


Unusual Observations for Na

Obs       Na      Fit  SE Fit  Residual  St Resid
  4  13.3200  12.3274  0.4313    0.9926      2.30 R

R denotes an observation with a large standardized residual.


Least Squares Means for Na

treatment     Mean
1           0.0302
2           4.9890
3           8.3043
4          12.0800


Grouping Information Using Tukey Method and 99.0% Confidence

treatment  N  Mean  Grouping
4          3  12.1  A
3          3   8.3    B
2          3   5.0      C
1          3   0.0        D

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable Na
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

treatment  Lower  Center   Upper  --------+---------+---------+--------
2          2.483   4.959   7.435      (-----*------)
3          5.798   8.274  10.750             (------*-----)
4          9.574  12.050  14.526                    (-----*-----)
                                  --------+---------+---------+--------
                                        4.0       8.0      12.0


treatment = 2  subtracted from:

treatment   Lower  Center  Upper  --------+---------+---------+--------
3          0.8396   3.315  5.791  (-----*-----)
4          4.6153   7.091  9.567           (-----*-----)
                                  --------+---------+---------+--------
                                        4.0       8.0      12.0


treatment = 3  subtracted from:

treatment  Lower  Center  Upper  --------+---------+---------+--------
4          1.300   3.776  6.251   (-----*------)
                                 --------+---------+---------+--------
                                       4.0       8.0      12.0
```

Tukey Simultaneous Tests
Response Variable Na
All Pairwise Comparisons among Levels of treatment
treatment = 1 subtracted from:

treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	4.959	0.4980	9.957	0.0003
3	8.274	0.4980	16.613	0.0000
4	12.050	0.4980	24.194	0.0000

treatment = 2 subtracted from:

treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	3.315	0.4980	6.657	0.0023
4	7.091	0.4980	14.238	0.0000

treatment = 3 subtracted from:

treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	3.776	0.4980	7.581	0.0011

Calculation of Mineral Content

Treatment Level 2 Powders:

$$4.98\frac{\text{mg}}{\text{g}}\text{Na} \times \frac{1\text{g}}{10^3\text{mg}} = 0.00498 \times 100 = 0.498\ \%\ \text{Na}$$

100 g of this MPC would be expected to contain 0.498 g Na; therefore:

$$0.498\text{g Na} \times \frac{\text{mol}}{22.99\text{g}} = 0.021\ \text{mol Na per 100 g powder}$$

there exists 0.021 mol per 84.17g protein; per 100 g protein:

$$\frac{0.021\ \text{mol}}{84.19\ \text{g}} = \frac{x}{100\ \text{g}}$$

x = 0.026 mol Na

Treatment Level 3 Powders:

$$8.30\frac{\text{mg}}{\text{g}}\text{Na} \times \frac{1\text{g}}{10^3\text{mg}} = 0.0083 \times 100 = 0.83\ \%\ \text{Na}$$

100 g of this MPC would be expected to contain 0.83 g Na; therefore:

$$0.83\text{g Na} \times \frac{\text{mol}}{22.99\text{g}} = 0.036\ \text{mol Na per 100 g powder}$$

there exists 0.036 mol per 81.59g protein; per 100 g protein:

$$\frac{0.036\ \text{mol}}{81.59\ \text{g}} = \frac{x}{100\ \text{g}}$$

x = 0.044 mol Na

Treatment Level 4 Powders:

$$12.08\frac{\text{mg}}{\text{g}}\text{Na} \times \frac{1\text{g}}{10^3\text{mg}} = 0.01208 \times 100 = 1.208\ \%\ \text{Na}$$

100 g of this MPC would be expected to contain 1.208 g Na; therefore:

$$1.208\text{g Na} \times \frac{\text{mol}}{22.99\text{g}} = 0.0525\ \text{mol Na per 100 g powder}$$

there exists 0.0525 mol per 81.54g protein; per 100 g protein:

$$\frac{0.0525\ \text{mol}}{81.54\ \text{g}} = \frac{x}{100\ \text{g}}$$

x = 0.064 mol Na

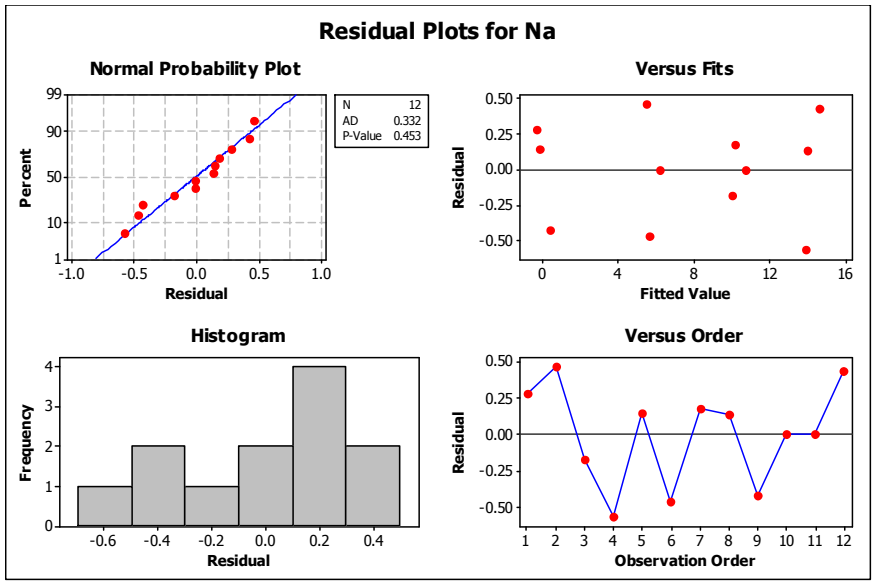


Figure 71: Residual plots for univariate ANOVA of Na content in dry MPC; Top Left: Normal probability plot (p = 0.453); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

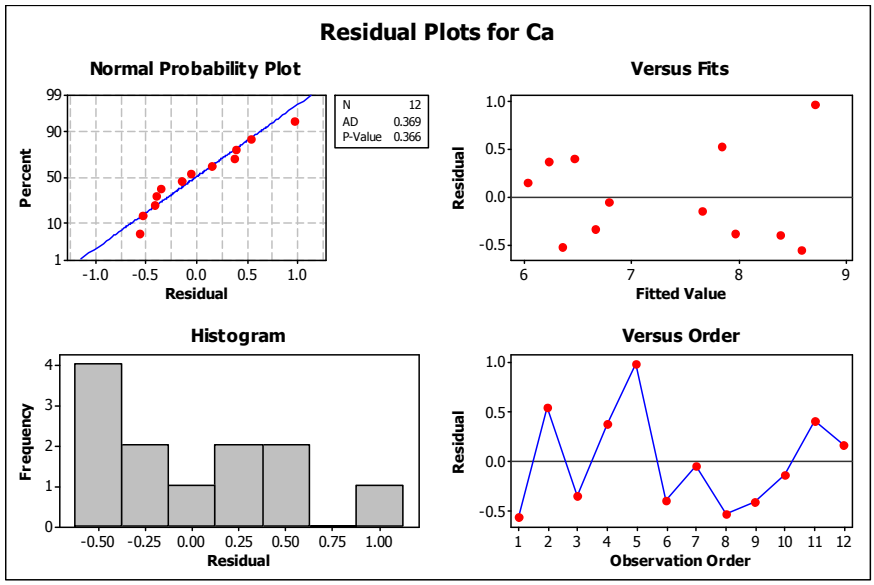


Figure 72: Residual plots for univariate ANOVA of Ca content in dry MPC; Top Left: Normal probability plot (p = 0.366); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

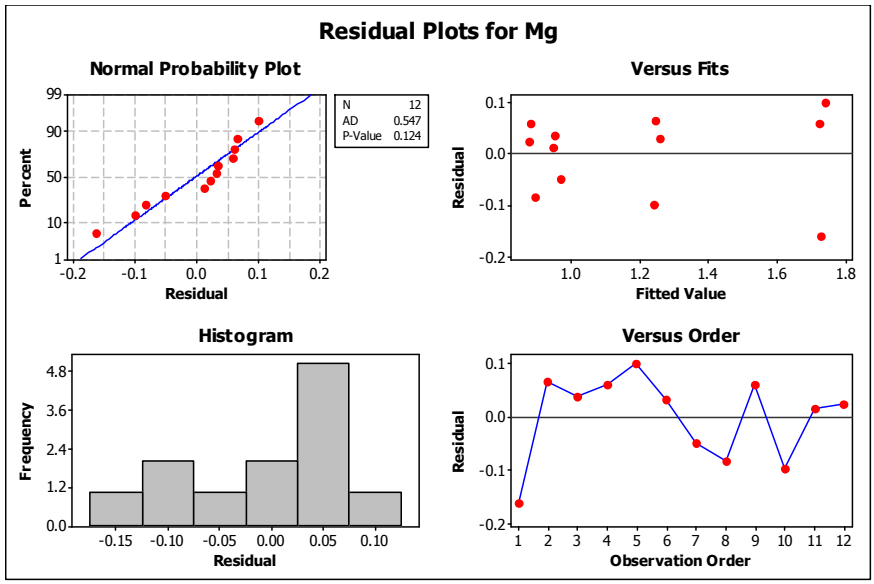


Figure 73: Residual plots for univariate ANOVA of Na content in dry MPC; Top Left: Normal probability plot (p = 0.124); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

Appendix O: Moisture Content of Dry MPC

Table 21: Moisture content of MPC powders as determined by AOAC 925.23

Sample	Block	Treatment	Moisture (%)
MPC	1	1	8.01
MPC	1	2	6.69
MPC	1	3	5.38
MPC	1	4	6.28
MPC	2	1	7.04
MPC	2	2	5.79
MPC	2	3	6.15
MPC	2	4	6.90
MPC	3	1	6.35
MPC	3	2	5.63
MPC	3	3	5.86
MPC	3	4	6.52

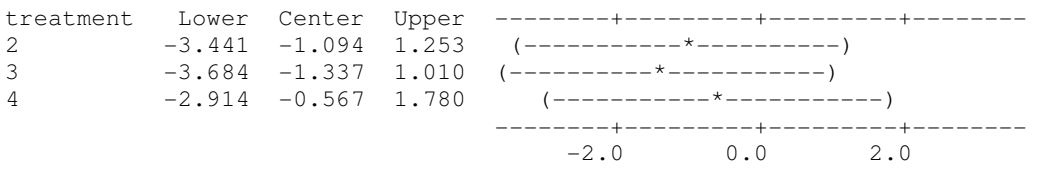
Minitab Output for Moisture Analysis of MPC powders

General Linear Model: moisture versus block, treatment

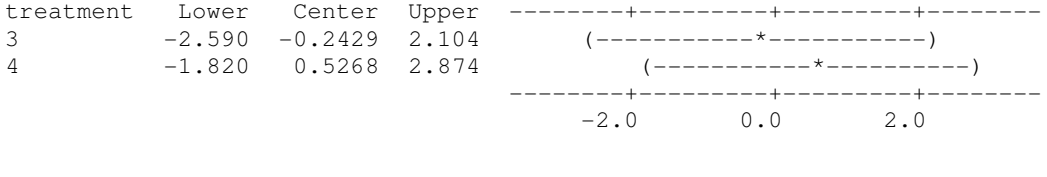
```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for moisture, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
block       2  0.5446  0.5446  0.2723  0.81  0.487
treatment   3  3.1759  3.1759  1.0586  3.17  0.107
Error       6  2.0064  2.0064  0.3344
Total      11  5.7268


S = 0.578275   R-Sq = 64.96%   R-Sq(adj) = 35.77%


Grouping Information Using Tukey Method and 99.0% Confidence

treatment  N  Mean  Grouping
1          3   7.1  A
4          3   6.6  A
2          3   6.0  A
3          3   5.8  A

Means that do not share a letter are significantly different.
```

Means that do not share a letter are significantly different.

Tukey 99.0% Simultaneous Confidence Intervals
Response Variable moisture
All Pairwise Comparisons among Levels of treatment
treatment = 1 subtracted from:



treatment = 2 subtracted from:



treatment = 3 subtracted from:

Tukey Simultaneous Tests
Response Variable moisture
All Pairwise Comparisons among Levels of treatment
treatment = 1 subtracted from:

treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	-1.094	0.4722	-2.317	0.1959
3	-1.337	0.4722	-2.831	0.1053
4	-0.567	0.4722	-1.201	0.6481

treatment = 2 subtracted from:

treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	-0.2429	0.4722	-0.5145	0.9526
4	0.5268	0.4722	1.1158	0.6941

treatment = 3 subtracted from:

treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	0.7698	0.4722	1.630	0.4301

Appendix P: Particle Size of Dry Powder

Table 22: Measured particle size of MPC powders. Means and standard deviations were obtained from triplicate measurements.

Block	Treatment	Mean	Standard Deviation	d_{10}	d_{50}	d_{90}
1	1	133.00	117.10	49.50	104.70	218.80
1	2	76.41	78.35	29.06	65.25	109.70
1	3	158.20	184.90	39.15	92.50	448.90
1	4	135.00	163.40	36.16	82.72	322.50
2	1	81.79	40.65	34.36	78.81	130.50
2	2	89.25	65.36	33.43	79.87	142.50
2	3	98.42	109.80	29.08	68.31	170.20
2	4	89.28	89.89	30.27	69.01	136.20
3	1	109.80	98.57	39.42	89.06	174.50
3	2	171.10	213.70	45.78	100.4	413.30
3	3	116.80	134.50	32.52	79.39	200.40
3	4	83.18	69.680	29.14	71.81	135.10

Minitab Output for Particle Size Distribution Analysis (d90) of Dry MPC

General Linear Model: d90 versus Block, Treatment

```
Factor     Type    Levels  Values
Block      random       3  1, 2, 3
Treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for d90, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
Block       2   35031   35031   17516  1.11  0.388
Treatment   3   16018   16018    5339  0.34  0.798
Error       6   94450   94450   15742
Total      11  145499


S = 125.466   R-Sq = 35.09%   R-Sq(adj) = 0.00%


Term         Coef  SE Coef      T      P
Constant   216.88    36.22   5.99  0.001
Block
1           58.09    51.22   1.13  0.300
2          -72.03    51.22  -1.41  0.209
Treatment
1          -42.28    62.73  -0.67  0.525
2            4.95    62.73   0.08  0.940
3           56.28    62.73   0.90  0.404


Unusual Observations for d90
```

Obs	d90	Fit	SE Fit	Residual	St Resid
10	413.300	235.775	88.718	177.525	2.00 R

R denotes an observation with a large standardized residual.

Least Squares Means for d90

Treatment	Mean
1	174.6
2	221.8
3	273.2
4	197.9

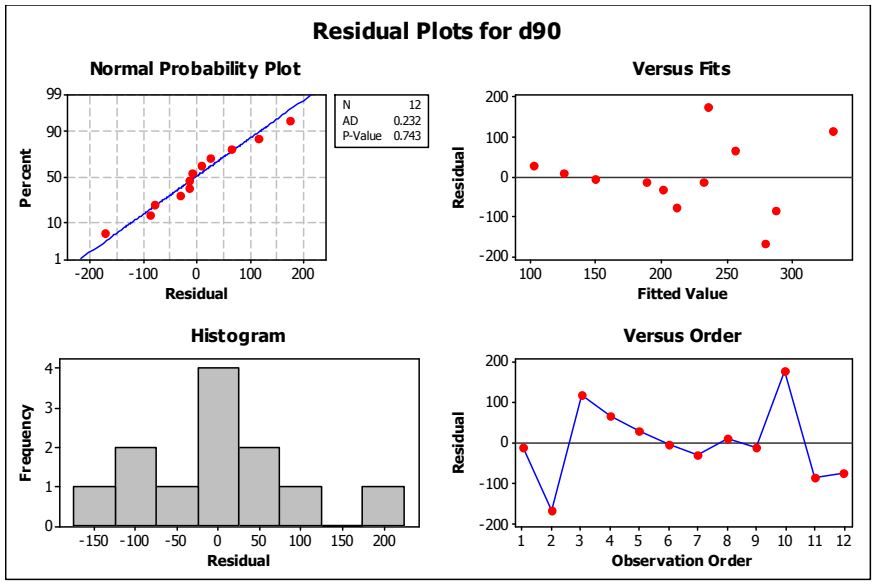


Figure 74: Residual plots for particle size distribution (d_{90}) as determined by Coulter LS 230; Top Left: Normal probability plot (p = 0.743); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

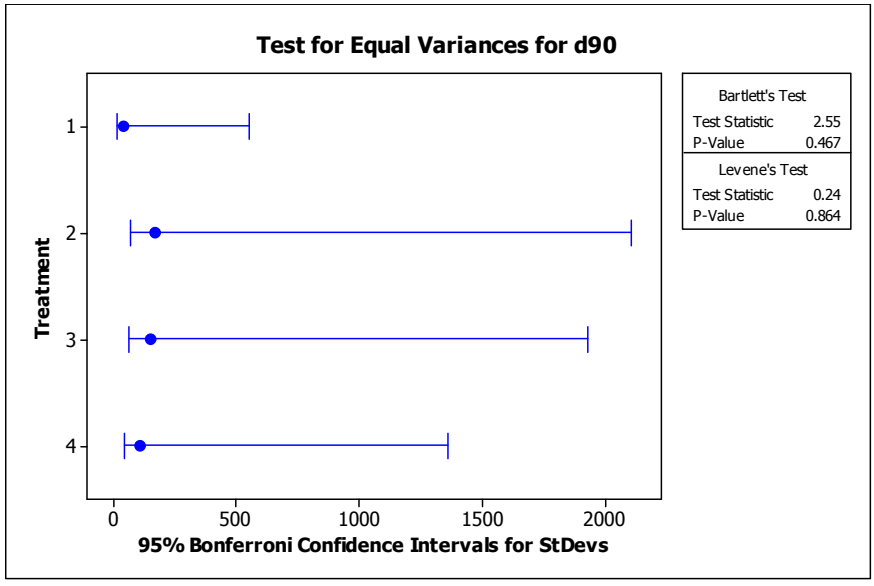


Figure 75: Plot of Variance for protein of dry MPC as determined by Elementar rapid N; Bartlett's Test statistic = 2.55, p-value = 0.467; Levene's Test statistic = 0.24, p-value = 0.864

General Linear Model: Mean versus Block, Treatment

```
Factor     Type    Levels  Values
Block      random       3  1, 2, 3
Treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for Mean, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
Block       2    3007    3007    1504  1.28  0.345
Treatment   3     782     782     261  0.22  0.878
Error       6    7064    7064    1177
Total      11   10853


S = 34.3121   R-Sq = 34.91%   R-Sq(adj) = 0.00%


Term          Coef  SE Coef      T      P
Constant   111.853    9.905  11.29  0.000
Block
1            13.80    14.01   0.99  0.363
2           -22.17    14.01  -1.58  0.165
Treatment
1            -3.66    17.16  -0.21  0.838
2             0.40    17.16   0.02  0.982
3            12.62    17.16   0.74  0.490


Unusual Observations for Mean

Obs     Mean      Fit  SE Fit  Residual  St Resid
  2   76.410  126.053  24.262   -49.643     -2.05 R
 10  171.100  120.621  24.262    50.479      2.08 R

R denotes an observation with a large standardized residual.


Least Squares Means for Mean

Treatment   Mean
1          108.2
2          112.3
3          124.5
4          102.5
```

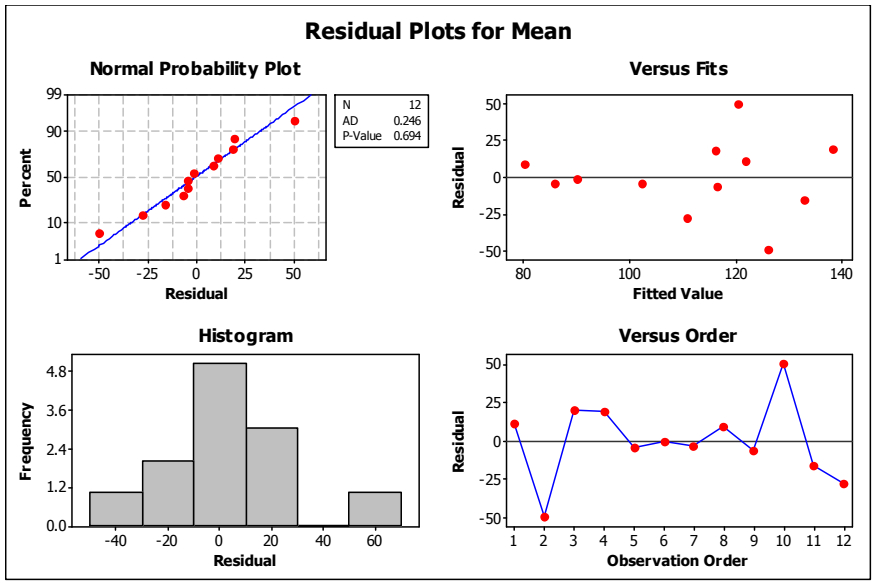


Figure 76: Residual plots for particle size distribution (mean size) of dry MPC as determined by Coulter LS 230; Top Left: Normal probability plot (p = 0.694); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

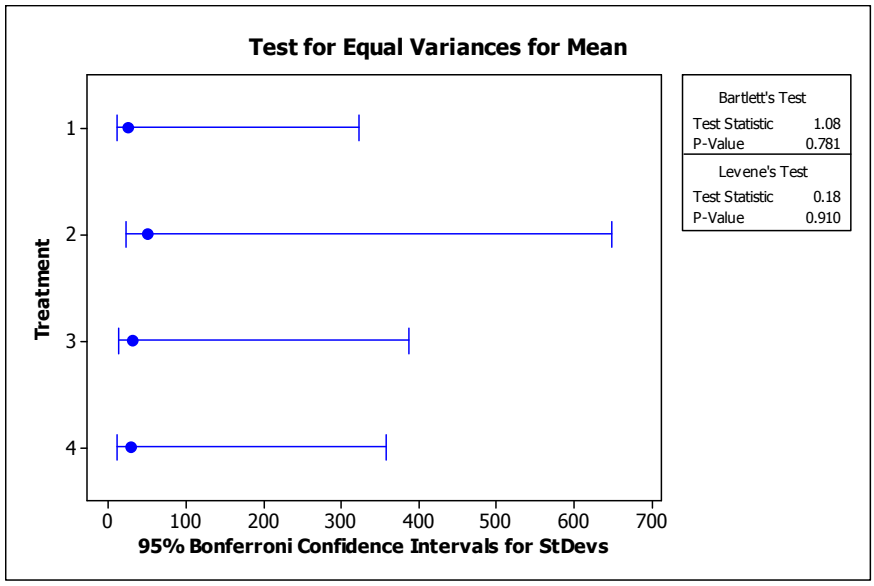


Figure 77: Plot of Variance for particle size distribution (mean size) of dry MPC as determined by Coulter LS 230; Bartlett's Test statistic = 1.08, p-value = 0.781; Levene's Test statistic = 0.18, p-value = 0.910

General Linear Model: d80 versus Block, Treatment

```
Factor     Type    Levels  Values
Block      random       3  1, 2, 3
Treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for d80, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS  Adj MS     F      P
Block       2   2622.6   2622.6  1311.3  3.01  0.124
Treatment   3  10346.3  10346.3  3448.8  7.92  0.017
Error       6   2612.7   2612.7   435.4
Total      11  15581.6


S = 20.8674   R-Sq = 83.23%   R-Sq(adj) = 69.26%


Least Squares Means for d80

Treatment    Mean
1          141.55
2          112.53
3           72.64
4           71.41


Grouping Information Using Tukey Method and 99.0% Confidence

Treatment  N   Mean  Grouping
1          3  141.6  A
2          3  112.5  A
3          3   72.6  A
4          3   71.4  A

Means that do not share a letter are significantly different.
```

Appendix Q: Particle Size of MPC During Reconstitution

Table 23: Measured particle size of particles during reconstitution. Means and standard deviations were obtained from duplicate measurements.

Block	Treatment	Mean	Standard Deviation	d_{10}	d_{50}	d_{90}
1	1	95.51	57.65	25.94	90.16	165.50
1	2	94.84	40.22	39.59	97.11	143.50
1	3	111.70	26.18	79.18	112.60	144.60
1	4	102.00	22.40	75.20	102.90	130.10
2	1	100.10	65.87	30.10	87.33	181.20
2	2	43.91	41.77	11.76	30.51	90.64
2	3	77.20	28.94	42.48	79.55	108.50
2	4	79.54	21.75	52.53	81.39	105.80
3	1	86.51	56.66	22.60	78.15	156.60
3	2	84.70	65.36	13.00	75.78	167.80
3	3	78.41	33.97	37.02	75.83	123.50
3	4	81.68	40.23	28.44	80.25	134.50

Minitab Output for Particle Size Distribution Analysis (Mean) of MPC During Reconstitution

General Linear Model: Mean versus Block, Treatment

```
Factor     Type    Levels  Values
Block      random       3  1, 2, 3
Treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for Mean, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
Block       2    3007    3007    1504  1.28  0.345
Treatment   3     782     782     261  0.22  0.878
Error       6    7064    7064    1177
Total      11   10853


S = 34.3121   R-Sq = 34.91%   R-Sq(adj) = 0.00%


Term          Coef  SE Coef      T      P
Constant   111.853    9.905  11.29  0.000
Block
1            13.80    14.01   0.99  0.363
2           -22.17    14.01  -1.58  0.165
Treatment
1            -3.66    17.16  -0.21  0.838
2             0.40    17.16   0.02  0.982
3            12.62    17.16   0.74  0.490
```

Unusual Observations for Mean

Obs	Mean	Fit	SE Fit	Residual	St Resid
2	76.410	126.053	24.262	-49.643	-2.05 R
10	171.100	120.621	24.262	50.479	2.08 R

R denotes an observation with a large standardized residual.

Least Squares Means for Mean

Treatment	Mean
1	108.2
2	112.3
3	124.5
4	102.5

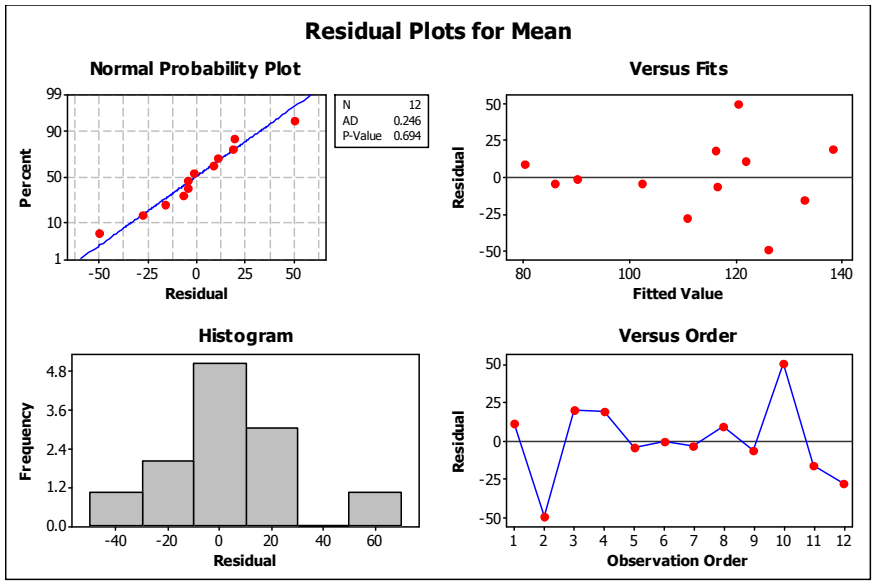


Figure 78: Residual plots for particle size distribution (mean size) of dry MPC as determined by Coulter LS 230; Top Left: Normal probability plot (p = 0.694); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

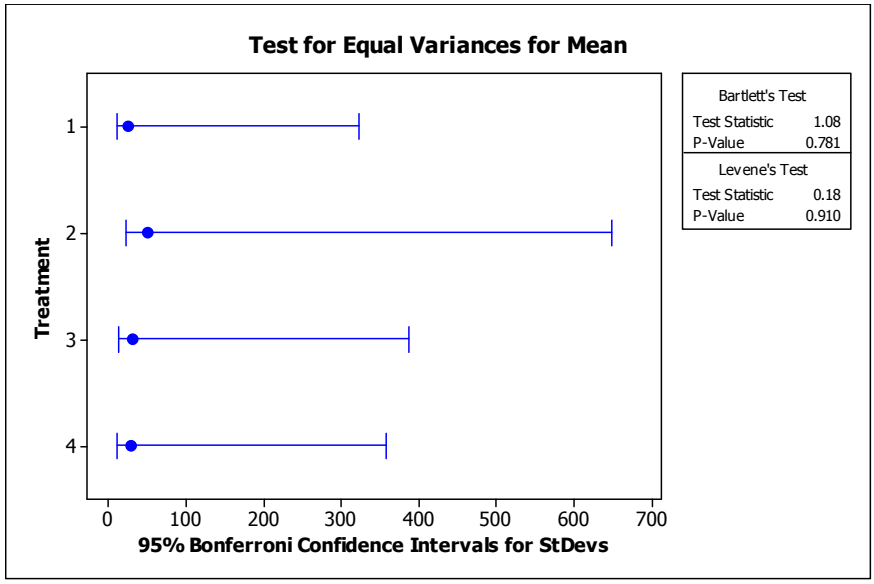


Figure 79: Plot of Variance for particle size distribution (mean size) of dry MPC as determined by Coulter LS 230; Bartlett's Test statistic = 1.08, p-value = 0.781; Levene's Test statistic = 0.18, p-value = 0.910

Minitab Output for Particle Size Distribution Analysis (d90) of MPC During Reconstitution

General Linear Model: d90 versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for d90, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
block       2  1565.5  1565.5   782.7  1.57  0.283
treatment   3  3805.4  3805.4  1268.5  2.54  0.152
Error       6  2993.2  2993.2   498.9
Total      11  8364.1


S = 22.3355   R-Sq = 64.21%   R-Sq(adj) = 34.39%

Least Squares Means for d90

treatment   Mean
1          167.8
2          134.0
3          125.5
4          123.5


Tukey Simultaneous Tests
Response Variable d90
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
2              -33.79       18.24   -1.853    0.3373
3              -42.23       18.24   -2.316    0.1962
4              -44.30       18.24   -2.429    0.1712


treatment = 2  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
3               -8.45       18.24  -0.4632    0.9645
4              -10.51       18.24  -0.5765    0.9356


treatment = 3  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
4              -2.067       18.24  -0.1133    0.9994
```

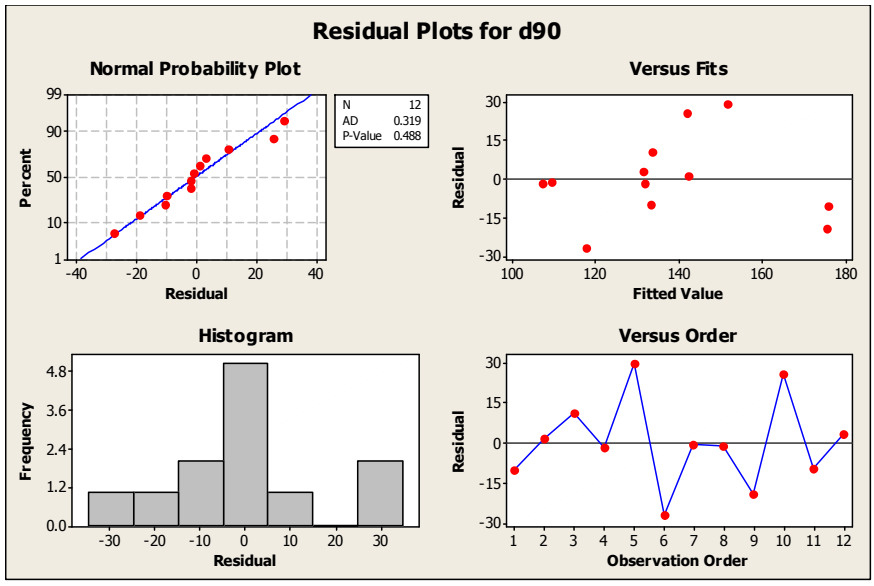


Figure 80: Residual plots for particle size distribution (d_{90}) of MPC during reconstitution as determined by Coulter LS 230; Top Left: Normal probability plot (p = 0.488); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

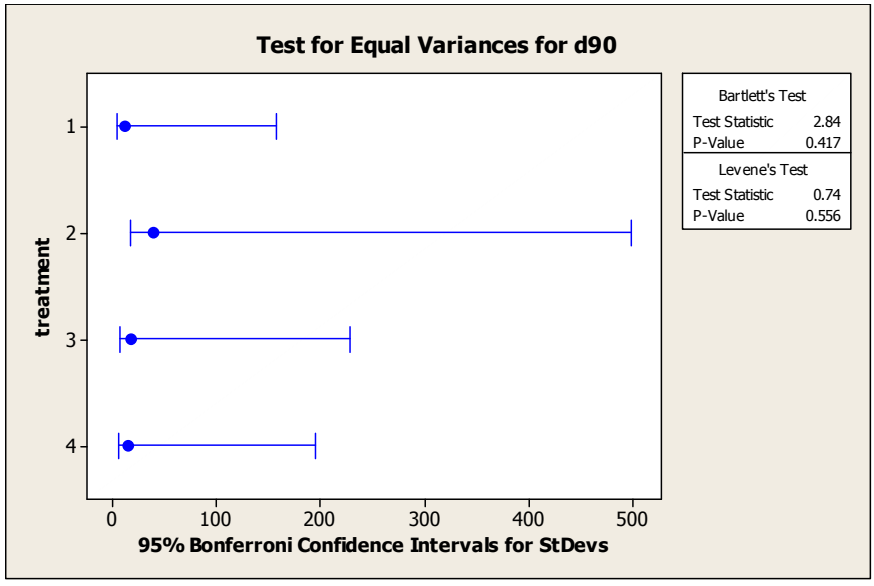


Figure 81: Plot of Variance for particle size distribution (d_{90}) of dry MPC as determined by Coulter LS 230; Bartlett's Test statistic = 2.84, p-value = 0.417; Levene's Test statistic = 0.74, p-value = 0.556

Minitab Output for Particle Size Distribution Analysis (Mean) of MPC During Reconstitution

General Linear Model: mean versus block, treatment

```
Factor     Type    Levels  Values
block      random       3  1, 2, 3
treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for mean, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS     F      P
block       2  1408.1  1408.1   704.0  3.48  0.099
treatment   3   628.4   628.4   209.5  1.03  0.442
Error       6  1215.0  1215.0   202.5
Total      11  3251.4


S = 14.2301   R-Sq = 62.63%   R-Sq(adj) = 31.49%

Least Squares Means for mean

treatment   Mean
1          94.04
2          74.48
3          89.10
4          87.74


Tukey Simultaneous Tests
Response Variable mean
All Pairwise Comparisons among Levels of treatment
treatment = 1  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
2             -19.56       11.62   -1.683    0.4066
3              -4.94       11.62   -0.425    0.9721
4              -6.30       11.62   -0.542    0.9453


treatment = 2  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
3              14.62       11.62    1.258    0.6173
4              13.26       11.62    1.141    0.6806


treatment = 3  subtracted from:

           Difference       SE of           Adjusted
treatment    of Means  Difference  T-Value   P-Value
4             -1.363       11.62  -0.1173    0.9994
```

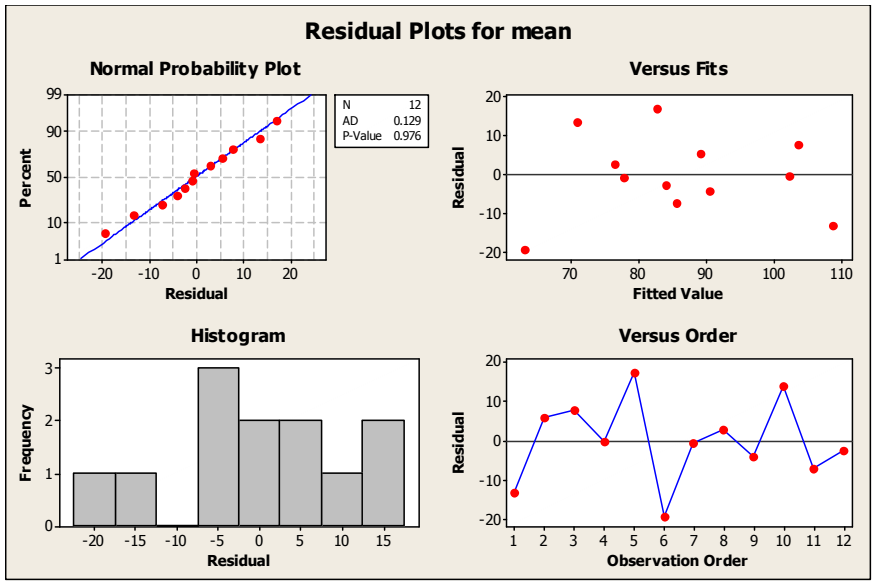


Figure 82: Residual plots for particle size distribution (mean) of MPC during reconstitution as determined by Coulter LS 230; Top Left: Normal probability plot (p = 0.976); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

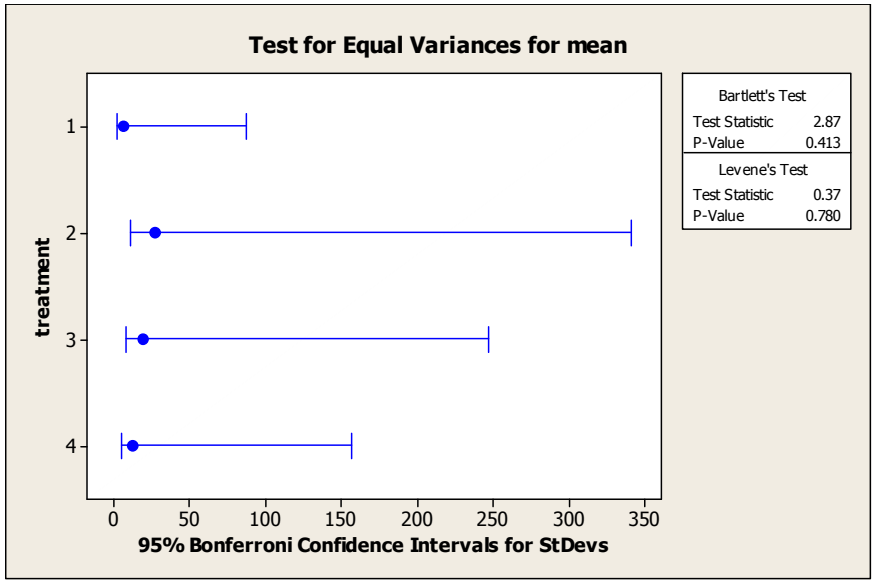


Figure 83: Plot of Variance for particle size distribution (d_{90}) of dry MPC as determined by Coulter LS 230; Bartlett's Test statistic = 2.87, p-value = 0.413; Levene's Test statistic = 0.37, p-value = 0.780

Minitab Output for Particle Size Distribution Analysis (d80) of MPC During Reconstitution

General Linear Model: d80 versus Block, Treatment

```
Factor     Type    Levels  Values
Block      random       3  1, 2, 3
Treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for d80, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS  Adj MS     F      P
Block       2   2622.6   2622.6  1311.3  3.01  0.124
Treatment   3  10346.3  10346.3  3448.8  7.92  0.017
Error       6   2612.7   2612.7   435.4
Total      11  15581.6


S = 20.8674   R-Sq = 83.23%   R-Sq(adj) = 69.26%


Least Squares Means for d80

Treatment    Mean
1          141.55
2          112.53
3           72.64
4           71.41


Grouping Information Using Tukey Method and 99.0% Confidence

Treatment  N   Mean  Grouping
1          3  141.6  A
2          3  112.5  A
3          3   72.6  A
4          3   71.4  A

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable d80
All Pairwise Comparisons among Levels of Treatment
Treatment = 1  subtracted from:

Treatment   Lower  Center  Upper   --+---------+---------+---------+----
2          -113.7  -29.02  55.67         (-----------*-----------)
3          -153.6  -68.91  15.78   (-----------*-----------)
4          -154.8  -70.14  14.55   (-----------*-----------)
                                   --+---------+---------+---------+----
                                  -140       -70         0        70


Treatment = 2  subtracted from:

Treatment   Lower  Center  Upper   --+---------+---------+---------+----
3          -124.6  -39.89  44.81       (-----------*-----------)
4          -125.8  -41.12  43.58       (-----------*-----------)
                                   --+---------+---------+---------+----
```

```
                                  -140       -70         0        70


Treatment = 3  subtracted from:

Treatment   Lower  Center  Upper   --+---------+---------+---------+----
4         -85.93  -1.230  83.47             (-----------*-----------)
                                   --+---------+---------+---------+----
                                  -140       -70         0        70


Tukey Simultaneous Tests
Response Variable d80
All Pairwise Comparisons among Levels of Treatment
Treatment = 1  subtracted from:

           Difference       SE of           Adjusted
Treatment    of Means  Difference  T-Value   P-Value
2              -29.02       17.04   -1.703    0.3978
3              -68.91       17.04   -4.045    0.0260
4              -70.14       17.04   -4.117    0.0241


Treatment = 2  subtracted from:

           Difference       SE of           Adjusted
Treatment    of Means  Difference  T-Value   P-Value
3              -39.89       17.04   -2.341    0.1903
4              -41.12       17.04   -2.413    0.1745


Treatment = 3  subtracted from:

           Difference       SE of            Adjusted
Treatment    of Means  Difference   T-Value   P-Value
4              -1.230       17.04  -0.07219    0.9998
```

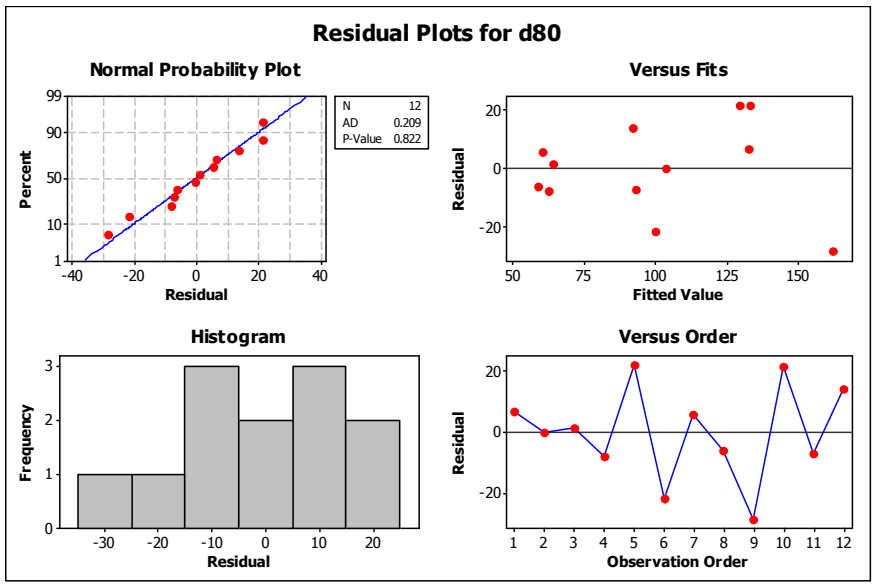


Figure 84: Residual plots for particle size distribution (d_{80}) of dry MPC as determined by Coulter LS 230; Top Left: Normal probability plot (p = 0.822); Top Right: Residuals versus fitted values; Bottom Left: Histogram of Residuals; Bottom Right: Residuals versus order

Minitab Output for Particle Size Distribution Analysis (d90-d50) of MPC During Reconstitution

General Linear Model: d90-d50 versus Block, Treatment

```
Factor     Type    Levels  Values
Block      random       3  1, 2, 3
Treatment  fixed        4  1, 2, 3, 4


Analysis of Variance for d90-d50, using Adjusted SS for Tests

Source     DF  Seq SS  Adj SS  Adj MS      F      P
Block       2  1107.7  1107.7   553.8   3.57  0.095
Treatment   3  4877.9  4877.9  1626.0  10.49  0.008
Error       6   930.3   930.3   155.0
Total      11  6915.9


S = 12.4516   R-Sq = 86.55%   R-Sq(adj) = 75.34%


Grouping Information Using Tukey Method and 99.0% Confidence

Treatment  N  Mean  Grouping
1          3  82.6  A
2          3  66.2  A
3          3  36.2  A
4          3  35.3  A

Means that do not share a letter are significantly different.


Tukey 99.0% Simultaneous Confidence Intervals
Response Variable d90-d50
All Pairwise Comparisons among Levels of Treatment
Treatment = 1  subtracted from:

Treatment   Lower  Center   Upper  ----+---------+---------+---------+--
2          -66.91  -16.37  34.165         (------------*------------)
3          -96.88  -46.35   4.192  (------------*------------)
4          -97.80  -47.27   3.272  (------------*------------)
                                  ----+---------+---------+---------+--
                                    -80       -40         0        40


Treatment = 2  subtracted from:

Treatment   Lower  Center  Upper  ----+---------+---------+---------+--
3          -80.51  -29.97  20.56      (------------*-----------)
4          -81.43  -30.89  19.64      (------------*------------)
                                   ----+---------+---------+---------+--
                                     -80       -40         0        40


Treatment = 3  subtracted from:

Treatment   Lower   Center  Upper  ----+---------+---------+---------+--
4          -51.46  -0.9200  49.62             (------------*-----------)
                                   ----+---------+---------+---------+--
```

-80 -40 0 40

Tukey Simultaneous Tests
Response Variable d90-d50
All Pairwise Comparisons among Levels of Treatment
Treatment = 1 subtracted from:

Treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
2	-16.37	10.17	-1.610	0.4392
3	-46.35	10.17	-4.559	0.0151
4	-47.27	10.17	-4.649	0.0138

Treatment = 2 subtracted from:

Treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	-29.97	10.17	-2.948	0.0915
4	-30.89	10.17	-3.039	0.0822

Treatment = 3 subtracted from:

Treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	-0.9200	10.17	-0.09049	0.9997